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NEWER ASPECTS OF THE ALVEOLAR STRUCTURE OF PROTOPLASM[1]

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AT this day we no longer need the admonition that we must maintain a critical attitude towards all conceptions of protoplasm based on the study of fixed and stained sections—that is to say, on the study of cells that have been subjected to the barbarous insults of coagulation, dehydration, cooking in hot paraffin, embedding, sectioning, staining and mounting in Canada balsam. Our faith in the results obtained by the practice of this kind of black magic is now less confiding than it was in a younger and more credulous age. If we do not tread warily amid the pitfalls of cytological technique it is not for lack of warning, though I must admit that even now that warning is not always sufficiently heeded.

But there is another side to this question. In this more sophisticated era, as our attention is more and more taken up with studies on living protoplasm, some danger may perhaps lurk in too pious a faith in methods that cut loose altogether from cytological technique or even from the results obtained by its troublesome practice. I resist the temptation here to homiletic discourse beyond the remark that there is no royal road to a solution of the complex and difficult problem before us. It is a business that calls for a union of all our forces and

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ways of approach. This is well attested, I think, by the history of opinion concerning what Bütschli called the foam-structure or alveolar structure of protoplasm (*Schaumstruktur, Wabenstruktur*). Peculiar advantages for the study of this type of structure are offered by the transparent eggs of certain echinoderms; and probably in no other object has the structure and behavior of such protoplasm more often been examined. I propose to discuss this subject from the standpoint of researches, especially on the eggs of sea-urchins and starfish, that I began more than thirty years ago and have recently again taken up, both by the study of living eggs and by the use of the modern technique for the formed components of the cytoplasm. Though incomplete, these studies show that we still have much to learn in this direction.

It should be said at once that in some of its more general and theoretical aspects Bütschli's account of protoplasm now seems almost obsolete, though it still retains a certain utility as a convenient and non-committal description of certain conditions that often occur. The so-called alveolar theory nevertheless is of enduring significance because it led the way in a reaction against conceptions of protoplasm derived from the study of coagulated cells. Based originally on the study of living cells, it was a successful attempt to show how a relatively stable, visible framework can exist in a protoplasmic system that has the general properties of a viscid liquid. Bütschli, however, enlarged his conception by the study of fixed and stained sections, and by experiments with oil-emulsions that served him as physical models for the structure, and, to a certain extent also for the activities, of actual protoplasm. His work thus contributed in a large way to the investigation of protoplasm regarded as a colloidal system; and it deserves a high place in the history of cytology, even though some of his conclusions have now been outgrown.

The fact should be emphasized that Bütschli's so-called "theory" did not deal primarily with the question

of an ultra-microscopical structure in protoplasm, though it is of great interest for this side of the problem. It was offered as an objective description of the visible or microscopical structure; and it is only this structure with which the following discussion will deal. The main outlines of his conclusions are known to everyone. I do not wish to dwell upon an old story; but it will conduce to clearness to recall its main features. Bütschli described protoplasm as a visible aggregate of two non-miscible, more or less viscid, liquids in emulsion-like association—in other words, as a diphasic system in which the continuous phase is represented by the ground-substance or hyaloplasm, the disperse phase by visible suspended bodies or droplets of enchylema. These bodies he often called "alveoli," a rather inappropriate term which has now generally been superseded by other names, such as alveolar spheres or (in case of the sea-urchin egg), macrosomes in contradistinction to certain much smaller dispersed bodies or "microsomes" scattered among them. The alveolar spheres sometimes remain spheroidal, but often become closely crowded and flattened together so as to assume polyhedral forms. In the latter case the hyaloplasm is reduced to a meshwork or framework of delicate films between them.

Whatever estimate we may place upon Bütschli's theoretical conclusions, it is not open to doubt that protoplasm often shows the structure described by him. I digress for a moment to speak of the question, sometimes raised even to-day, as to whether the dispersed formed components of the system should be included in the conception of protoplasm, or whether the latter term should be reserved for the hyaloplasm alone. Bütschli himself trod cautiously on this treacherous ground, though passages might be cited from his works that would seem to favor both alternatives. In one significant paragraph, however, he says: "As long as individual constituents of the cell are not seen to persist when isolated . . . it is very dangerous to speak of their life as something which they

possess in themselves. They are living in so far as they are parts of a living organism." Here he comes close to the view, earlier expressed by Flemming and later adopted by many more modern investigators, that we can not restrict the term protoplasm to any single substance or component of the cell. Opinion on this subject is, however, not yet unanimous. Among both cytologists and physiologists there can still be found some workers who seem to think of the hyaloplasm or continuous substance as the fundamental living stuff or protoplasm, and for this reason have spoken of protoplasm as "structureless."[1] Such a conception of protoplasm, however, can not, I think, be logically justified. In point of fact, most modern investigators, including physiologists and biochemists as well as cytologists, now hold that life is the sum-total of activities in the cell-system and that it is not at present possible to exclude from the conception of the living stuff or protoplasm the substance of the dispersed or formed bodies. As has often been urged, accordingly, the word protoplasm does not designate a single substance. It is not a chemical term but a morphological one, a collective name for the sum-total of the active components that cooperate in the work of a complex system.

We still know comparatively little, it is true, concerning the activities of the formed bodies of the cytosome. In spite of the recent marked revival of interest in these bodies we are on the whole astonishingly ignorant in regard to them. But the day has gone by when it was possible to wave them all airily aside as needless luxuries or useless by-products. If any of us are still disposed to dispute their claims to biological respectability we might with advantage reflect on the activities of the plastids in photosynthesis and other chemical work of the cell, or

[1] It is this tendency, I suppose, that explains the frequent designation of the formed bodies as "cytoplasmic inclusions"—a phrase that deserves to stand beside that other rather entertaining one, "Cytology, including Cell-contents," which long has stood as a heading for the cytological reviews in a well-known and valued biological journal.

on those of the Golgi-bodies in the production of secretory granules and possibly of the organic enzymes generally. The chromosomes, after long wanderings in the wilderness, seem at last to have obtained a secure footing inside the pale. The central bodies are not far behind them; and a good word may even be spoken for the chondriosomes, though their character is not yet above reproach and there are sinister rumors that their claim to membership in the cell-community may be illegitimate. In any case we have enough reason to look skeptically upon any attempt to restrict the operations of life (and hence to restrict the term protoplasm) to the continuous substance or hyaloplasm.

But, to return to Bütschli, I must mention two other conclusions of his that are of importance for my discussion, though both, I believe, have been shown to be untenable. First, he drew always a sharp distinction between a so-called primary, true or finer structure and a secondary, false or coarser one. The first of these he considered as a universal characteristic of protoplasm, the second as a variable, inconstant and derived one arising by the frequent deposit of larger spheroidal vacuoles, drops or granules which, if closely crowded, may offer a magnified *simulacrum* of the true structure. The "pseudo-alveolar structrue" thus arising is exemplified by the close crowding of yolk-spheres in many kinds of ova or of the secretory granules in gland-cells. Bütschli later broadened this distinction by characterizing the true structure as "positive," the false as "negative," according to the relative refractive index of its components, the alveolar spheres being in the first case watery drops of lower density and refractive index than the hyaloplasm, while the reverse condition prevails in the second case, irrespective of the size of the dispersed bodies. These distinctions are important here because the sea-urchin egg was included by Bütschli with other examples of the true or finer structure; and as such it was also described by various later observers. Some writ-

ers, such as Reinke or Meves, have, it is true, insisted that the ooplasm of the sea-urchin egg shows only a pseudo-alveolar structure; but perhaps we may assume that Bütschli was the most competent witness as to his own meaning; and the fact that dispute concerning this point could have arisen at all shows how theoretical, not to say transcendental, the whole distinction really is.

Secondly, Bütschli considered the visible alveolar structure as a general characteristic of protoplasm. Even in case of the so-called structureless or hyaline protoplasm he believed the alveolar spheres to be present, in the same form and of the same dimensions as in the visible type, but lost to view because of their close crowding and the consequent extreme tenuity of the interalveolar films. This view never became widely current; but apart from this, further investigation proved that the whole conception of alveolar structure as an original and universal property of protoplasm could not be maintained.

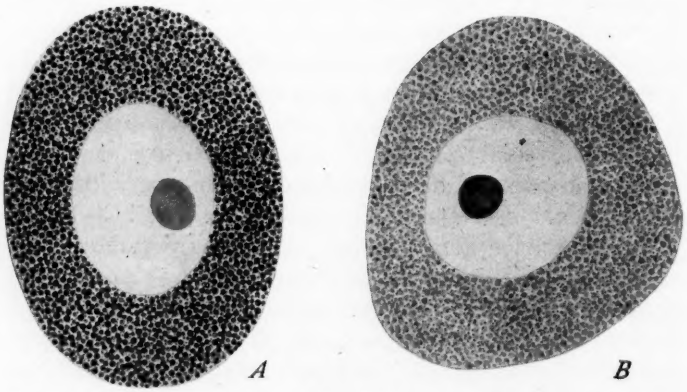

FIG. 1. *Full-grown oöcytes, Benda fixation. A, intensely stained with thionin; B, Kull method, showing yolk-spheres (pale blue) and small scattered mitochondria (red).

* All the figures, excepting Fig. 2, from sections of the sea-urchin *Arbacia punctulata*, 2–5 μ in thickness; Fig. 4 enlarged 1,040 diameters, the others 850 diameters.

In an earlier paper (1899), based on the study of sea-urchin eggs, and others, both living and in sections, I tried to show, first, that no logical distinction can be drawn between a true and a false alveolar structure, and secondly, that the alveolar structure is here a secondary formation, the origin of which can be traced step by step in the growing oocyte. My recent reexamination of this material confirms this, but also shows that in respect to the origin of the alveolar structure my former account left much to be desired. Like those earlier observations, but still more clearly, my recent ones demonstrate that the alveolar spheres or "macrosomes" of these eggs can not be distinguished in any clearly definable way from the yolk-spheres of other eggs. They are in fact yolk-spheres of small size, as they were actually called by Oscar Hertwig and later by myself, Meves and some other observers; and they may be intensely stained by some of the same methods employed for the yolk-spheres of other eggs. Like the latter they are absent in the very young oocytes, and only in later stages does the alveolar structure gradually appear by the formation and close crowding of these and other dispersed formed bodies. In this respect the sea-urchin egg offers precisely the same problem as those involved in the growth of other eggs.

I turn now to some of the more significant details in the structure and growth of these eggs. The earlier accounts of the alveolar structure in the echinoderm egg recognized the presence of only two or three types of dispersed bodies, the alveolar spheres or macrosomes, the microsomes, and (in *Arbacia*) the chromatophores or pigment-granules. It is now evident that the system is considerably more complicated than this. In 1899 I stated that even in the mature egg, although the macrosomes are for the most part fairly uniform in size, all gradations may be seen among the dispersed bodies from the largest down to the smallest. This statement has been criticized as based on the examination of crushed

or dying eggs; but, as stated in the original paper, the size-gradation was seen in the normal as well as in the crushed eggs. This is borne out by recent studies on very thin sections of *Arbacia* eggs intensely stained with thionin or toluidin blue. Such preparations (Fig. 1, A) clearly demonstrate the size-gradation and in other respects offer an interesting picture very different from

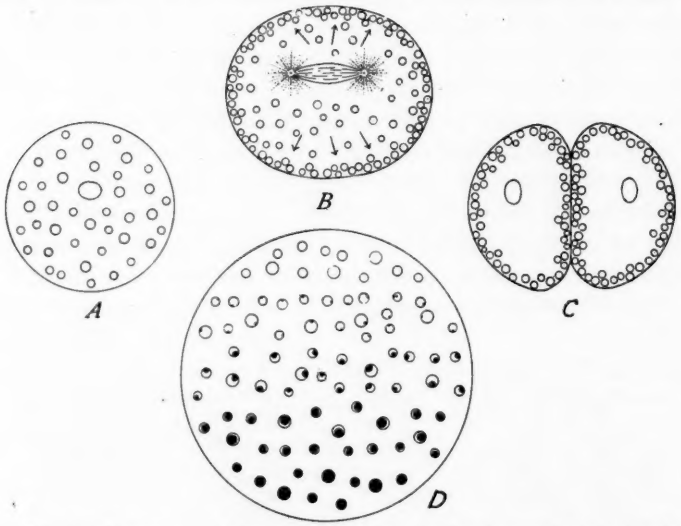

FIG. 2. Diagrams of living eggs showing only the chromatophores (much exaggerated in relative size). A, unfertilized egg; B, early first cleavage; C, 2-cell stage; D, living egg more enlarged, stained with Janus Green B. Successive stages of the action shown from above downwards. Actually all the chromatophores stain at the same rate throughout the egg.

many of the figures in the literature, including my own earlier ones as well as those of Bütschli. The alveolar spheres do not here appear as clear, vacuole-like drops but as deep blue spheroidal granules, less closely crowded than in most of the existing figures (those of Meves excepted). Superficially viewed these granules appear fairly uniform in size. More closely studied they are seen to vary materially, while among the larger ones are scattered smaller and smaller ones that form a perfectly

graduated series down to the limit of microscopical vision. All these bodies, large and small alike, are stained deep blue and show no trace of qualitative differences. Nevertheless, it may be shown by various methods that in spite of their continuous size-gradation, the dispersed bodies include in *Arbacia* at least five, and possibly as many as seven, distinct morphological types, not including the smallest granules, some of which are no doubt coagulation-artifacts. Each type shows considerable variations of size; hence the appearance of a continuously graded series. Of these types only two are distinguishable as such in the living egg, namely, the alveolar spheres or yolk, and the chromatophores or pigment-granules. The so-called "microsomes" comprise at least two distinct types, as will presently be shown. The main characters of these various bodies may be indicated as follows:

(1) The yolk-spheres appear as pale, colorless bodies; they are but slightly darkened by osmic acid; and they do not stain readily in most dyes though, as above mentioned, they may by suitable manipulation be intensely colored.

(2) The chromatophores are in life at once distinguishable by their red color; but in sections they can not be stained at all by most dyes, appearing as clear, vacuole-like spaces. In life, on the other hand, they alone among the dispersed bodies of the cytosome are intensely stained by Janus Green B, assuming a deep blue-black color by a process that seems to be of undescribed type. This is shown in slightly schematized form (but without exaggerating the clearness of the effect) in Fig. 2. The action begins always at a *single,* sharply localized, minute area exactly at the periphery, which then steadily enlarges, always with definite circular outline, until the whole chromatophore is uniformly stained.[2] The chromatophores are the largest of the dispersed bodies and also the heaviest, as is readily seen in centrifuged eggs.

[2] See *Anat. Record,* XXXI, 4, 1925.

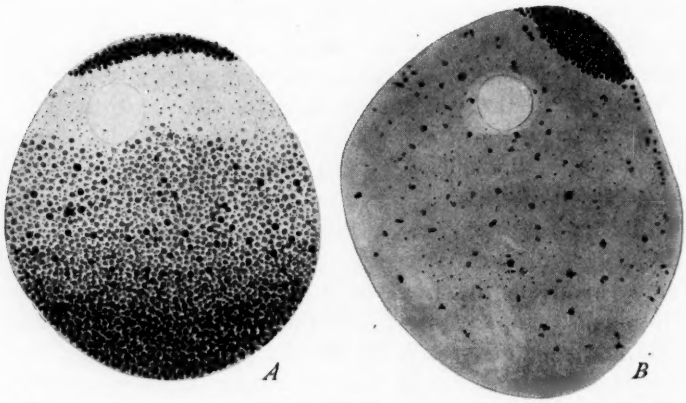

FIG. 3. Sections of mature centrifuged eggs. A, Benda-Kull method; B, Benda-osmic (Kolachev method). In A (slightly schematized) the yolk-spheres are pale blue, the oil-globules ("Golgi-yolk"), black, are massed at the light pole, the mitochondria red. The chromatophores, massed towards the heavy pole, colorless in the section are here made black.

(3) Chondriosomes. Mitochondria. By these names I will designate certain small granules scattered between the yolk-spheres, showing the same general characters as those described under the same name by Meves in *Sphaerechinus,* and belonging in the general category of "microsomes." In sections (Fig. 1, B) they are stained intensely by the standard chondriosome methods, such as those of Benda, Meves or Kull, and are thus sharply differentiated from the yolk-spheres. For this reason I am inclined to accept Meves's identification of them as chondriosomes in spite of the noteworthy fact that in the course of numerous experiments I have never seen them sharply stained *in vivo* by Janus Green B, nor have I ever seen them as rods, though they sometimes appear in the form of short chains, somewhat as figured by Meves.[3]

[3] It has been stated that the chondriosomes of the sea-urchin egg are rod-shaped, more abundant towards the periphery, and that they stain *in vivo* with Janus Green. My own experience in case of the sea-urchin has been negative in respect to all three points; but in the starfish (*Asterias*) there is a clearly marked peripheral zone of spheroidal mitochondria in addition to those scattered through the egg. (See "General Cytology," 1924, p. 245.)

(4) Oil-globules or "fatty yolk." These bodies, made familiar by the centrifuging experiments of Lyon and his successors, are intermediate in size between the yolk-spheres and the mitochondria and less numerous than either. They blacken intensely in osmic acid, but are completely bleached by subsequent exposure for eighteen hours or more to turpentine. In the latter respect they are readily differentiated from the Golgi-bodies as described below. In strongly centrifuged eggs, as has long been known, they form a sharply localized cap at the light pole (Fig. 3); the yolk-spheres mass in the heavy hemisphere with the chromatophores crowded around the corresponding pole; while the mitochondria are but slightly affected, though somewhat less numerous towards the light pole.

(5) There remains a group of bodies that undoubtedly includes the Golgi-apparatus or its products, though I have not yet been able to trace in full their history and

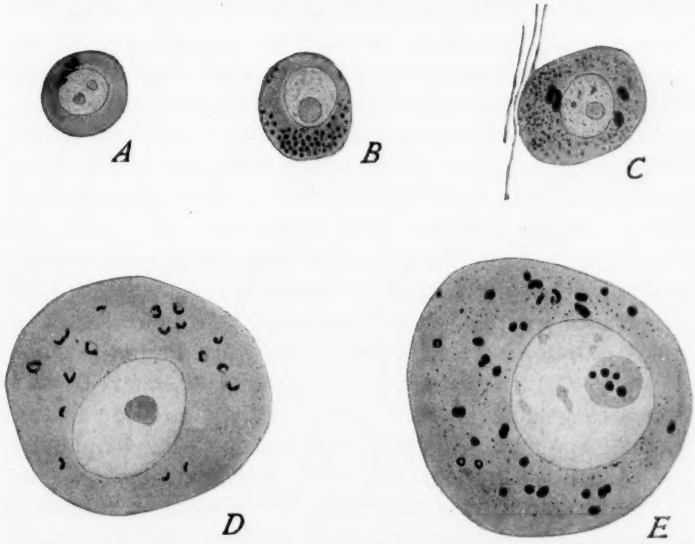

FIG. 4. Sections of earlier oöcytes. A, D, Champy-osmic, Golgi-platelets only; B, Champy-Kull; C, Benda method osmic; E, Benda-osmic, Golgi-bodies only.

relationships. They blacken intensely in osmic acid (*e.g.*, by the method of Kolatchev) but differ from the oil-globules in the fact that they are neither bleached by turpentine, even after several days' exposure, nor are they affected by the centrifuge. They are of several morphological types, including typical Golgi-platelets, remarkable ring-figures (probably optical sections of spheres), and smaller rounded granules of varying size, besides irregular forms. Typical Golgi-platelets, each apparently applied to a vaguely seen clear spheroid, are found in the earlier oocytes after Champy-osmic treatment (Fig. 4, A, D); but in these same stages after Benda-osmic they usually appear as black spheroids (Fig. 4, C, E) closely similar to the bodies that have been described in some other eggs as extruded nucleoli or nucleolar fragments. I can not, however, find the least valid evidence of such an origin. It seems more probable, since rings of corresponding size are also found in some of the same cells (Fig. 4, E), that the spheroids are Golgi-bodies in which the body on which the platelet is moulded, ordinarily colorless, has blackened, or in which the original platelet has extended completely around it.

Later oocytes, after Benda-osmic, sometimes show similar dark spheres, but more commonly rings or irregular bodies, often a few smaller crescents or platelets, and many smaller rounded black granules. The most remarkable of these forms are the rings (Fig. 5, A), which are very sharply defined, with transparent centers and deep black periphery, as if drawn in ink. They are not uncommonly associated in pairs, as if dividing by fission. Possibly they may belong to the chromatophores, with which they closely agree in size and distribution in the normal egg; but this possibility seems to be opposed by conditions in sections of centrifuged eggs, where the chromatophores appear as unstained, clear spaces crowded towards the heavy pole, while typical rings may be seen elsewhere, though never in large numbers. In some series of sections (Benda-osmic) rings are wanting,

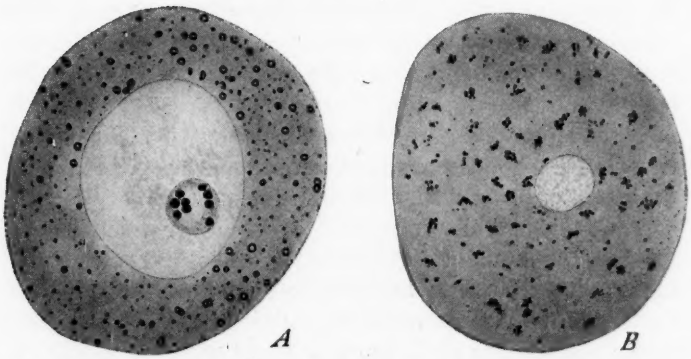

FIG. 5. Full grown oöcytes, before and after maturation. Benda-osmic. Golgi-bodies only.

but in their place appear small groups of rather uniform black granules, resistant to turpentine (Fig. 5, B) which perhaps arise by breaking up of the rings. The probable origin of the rings has earlier been indicated (p. 116).

To sum up: despite considerable uncertainty concerning the relations of the bodies in question it seems clear that these eggs, like many others, contain Golgi-bodies or their products, which appear in various forms scattered through the cytosome and contribute to the alveolar structure but are indistinguishable without the use of a special technique.

I turn now from the details to certain broader aspects of the subject. My observations add to the evidence, first, that the alveolar formation in these eggs represents a complicated aggregate comprising many components, diverse both chemically and physically. This fact, it seems to me, must be of importance for experimental studies on these eggs; and it is possible that the complication is much greater than thus far appears.

Secondly, these observations confirm in principle the conclusion that the visible alveolar structure is not a primary characteristic of the ooplasm but a secondary product of development. This is clearly shown by the

study both of living oocytes and of sections. The visible alveolar structure arises secondarily by the appearance and close crowding in the hyaloplasm of dispersed formed bodies.

Thirdly, however, it is now clear that my earlier account of this process was somewhat misleading in stating that "the entire alveolar structure is of secondary origin, arising through the formation of liquid or viscid drops in a sensibly homogeneous basis."[4] Insofar as this statement seemed to imply that all the dispersed bodies of the system may arise *de novo* and *in situ* in the hyaloplasm it can not now be maintained without material qualification. As above stated the Golgi-bodies, and apparently also the mitochondria, are present from a very early stage, not dispersed through the cytosome, but more or less massed towards one pole of the nucleus (Fig. 4) whence they later spread through the cytosome and increase in number, as has been described in other eggs. Their original source has not been determined, but there are many reasons to suspect that they may be received as such from the last generation of oogonia. Concerning the origin of the other components of the ooplasm I have thus far been unable to reach a definite conclusion. The oil-globules have been supposed in case of certain other eggs to be products of the Golgi-bodies and have accordingly been characterized as "Golgi-yolk" or "G-yolk."[5] To the alveolar spheres or yolk every possible source has been ascribed, including Golgi-bodies, chondriosomes, extruded nucleoli or nucleolar fragments, and formation *de novo* in the hyaloplasm. Concerning the difficult problems here involved I have no present conclusion to state; and the echinoderm egg is perhaps unfavorable for their solution. Thus far the only indubitable fact is that somehow the cytosome becomes crowded with yolk-spheres that do not at first exist as such.

The genesis and functional significance of the formed components of the ooplasm seem worthy of the most

[4] *Journ. Morph.*, XV, *Suppl.*, 1899, p. 11.

[5] *Cf.* Brambell, *Brit. Journ. Exp. Biol.*, I, 1.

careful further study with reference to the problems of development as well as of the protoplasmic activities generally. Studies on centrifuged eggs have thus far seemed, it is true, unfavorable to the possibility that the formed bodies may play an essential part in development; but I think this conclusion should not be unreservedly accepted without a more adequate cytological examination of such eggs. The question thus opened is, however, too large to be taken up here.

The problems involved in the alveolar structure of the ooplasm belong to the larger one of the significance and origin of cytoplasmic systems generally. All now seems to indicate that the visible alveolar structure in tissue-cells, alike of plants and of animals, is not a primary characteristic of protoplasm but rather an incidental consequence of the crowding together of rounded dispersed bodies, whether they be vacuoles or watery drops or bodies of more solid consistency. We know that these bodies may differ widely in respect to their size, grouping, physical and chemical nature and physiological significance, in different types of cells and in different physiological phases of the same cell. They may be widely scattered, massed in some regions and scanty or missing in others; and the so-called alveolar structure may thus vary endlessly or even disappear entirely.

I repeat, therefore, that visible alveolar formations, such as those described by Bütschli, are of only secondary significance for the problem of protoplasmic structure. They are but varying aspects, kaleidoscopic pictures, of heterogeneous systems that comprise a great variety of components, both formed and unformed. Some of these may be merely incidental and passive by-products, and as such may perhaps not properly belong to the living system; others undoubtedly play an essential part in its activities.

An interesting question, closely connected with the foregoing one and now prominently before cytologists, concerns the genetic relations of the cytoplasmic compo-

nents. In what measure may these components, whether formed or unformed, be genetically continuous from generation to generation (as is the case, for instance, with plastids and up to a certain point also with the central bodies) and in what measure may they arise anew in each cell-generation?[6] We know that some of them may be formed "*de novo*," *i.e.*, without direct relation to pre-existing bodies *of the same kind.* This, however, does not touch the root of the matter. The question still remains as to how far bodies thus produced may arise from, or be determined by, pre-existing bodies that are themselves self-propagating—as, for example, starch is formed by plastids, secretory granules by Golgi-bodies, and possibly other things by chondriosomes. This question, so important for the problems of differentiation as well as those of protoplasmic structure, is still widely open and calls for a suspension of judgment. Until it has been further elucidated our conception of cytoplasmic systems, and hence our use of the word protoplasm, must be of only provisional character. Meanwhile it is well to confess our present inability to give any universal formula for the structure of protoplasm. As Professor Harper has well said (1919), the structure of protoplasm is the structure of the cell. The remark has been called a platitude; but that may be taken as only an uncomplimentary way of saying that it is true.

[6] *Cf.* Wilson, AM. NAT., Nov., 1925.

THE ACTION OF ELECTROLYTES ON THE PHYSICAL STATE OF PROTOPLASM[1]

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THE realization that the colloidal state is one of the most important attributes of protoplasm must not blind one to the fact that protoplasm can not be regarded merely as a substance. It is a mechanism. The cell is the unit of structure of living matter, and protoplasm is protoplasm only insofar as it exists within the confines of a microscopic cell with its nucleus and its surrounding membranes. Crush a cell so as to destroy its morphologic structure and you destroy protoplasm. In attempting, therefore, to study the physical properties of protoplasm we must take into consideration the existence of its peculiar structural organization. This requires a new method of approach, for we can experiment with protoplasm only while it is intact as a living cell. A study of this nature is made possible by means of the microdissection and micro-injection apparatus which permits the manipulation and minute dissection of the living cell and the introduction of substances directly into the internal protoplasm. The dissection of cells is performed with delicate glass needles which taper to a point of excessive fineness. By means of the apparatus the movements of the needles can be accurately controlled within the field of the highest magnification of the microscope.

The essential nature of the limiting surface membrane or the plasmalemma of protoplasm is well shown on tearing the surface of a living cell. If the tear is made rapidly, a disintegration of the plasmalemma takes place which spreads from the site of injury over the entire body of the protoplasm, whereupon the denuded protoplasm at

[1] Address given at the symposium on "The Structure of Protoplasm," American Society of Naturalists, New Haven, Conn., December 30, 1925.

once scatters and dissipates throughout the environing medium. With a slow tear, however, the plasmalemma rapidly reforms and maintains the integrity of the protoplasm within. In this way, a cell can be cut into several pieces. As long as an intact plasmalemma surrounds a mass of protoplasm, the life activities of the protoplasm continue for a limited time if the nucleus is absent, and for a much longer time if the nucleus is present.

Even when we consider the homogeneous appearing cytoplasm within the plasmalemma, we still can find demonstrable differences in the physiologic behavior of different regions. This is shown in the following experiment on the mature starfish egg. If the surface of the egg is torn while the egg is under compression, the fluid interior can be made to ooze out through the tear as a droplet of protoplasm surrounded by a surface membrane. What is left behind is the collapsed, cortical portion of the egg. The cortical remnant is relatively solid but will eventually round up so as to appear like a diminutive egg. The material which has escaped from the egg immediately rounds up. The cortical remnant is readily fertilizable and undergoes normal segmentation. On the other hand, the material which has escaped from the interior of the egg, whether nucleated or not, is non-fertilizable. This experiment indicates the existence of a localized differentiation of certain physiologic properties in the cytoplasm. It is one proof that the intricacy of organization of protoplasm is expressed not only in the existence of a differentiated nucleus and a plasmalemma, but also within the homogeneous appearing cytoplasm itself.

One of the most important aspects of cell physiology is the rôle of electrolytes in the maintenance of protoplasmic structure and function. Considerable work has been done in the past and is being done now on the reaction of living cells to electrolytes, but it has not been possible to differentiate between the reaction of the exterior and interior of the cell except by means of

the micro-injection method. Micro-injection experiments were recently carried out with the collaboration of Dr. Paul Reznikoff on the protoplasm of the fresh water amoeba. Probably the most striking result of this study is the determination of specific differences between the monovalent and divalent cations in their action on the internal protoplasm and on the plasmalemma. When sodium chlorid and potassium chlorid are injected, the protoplasm becomes temporarily quiescent and liquefies, but subsequently tends to return to its normal state. Calcium chlorid and magnesium chlorid, on the other hand, solidify the internal protoplasm and the region solidified is usually irretrievably injured. The action of these salts on the plasmalemma are of a different order. Amoebae immersed in sodium chlorid soon become quiescent and the plasmalemma undergoes disintegration. The action of potassium chlorid is similar to that of sodium chlorid but to a lesser degree. In contrast to this, calcium chlorid and magnesium chlorid exert no appreciable effect on the amoeba as long as the plasmalemma remains intact. The order of toxicity of the salts on the interior of the cell is, therefore, just the reverse of that on its exterior; that is, when injected, calcium chlorid and magnesium chlorid are more toxic than potassium chlorid and sodium chlorid, whereas, in the immersion experiments, potassium chlorid and sodium chlorid are more toxic than calcium chlorid and magnesium chlorid. In both sets of experiments it has been found that the toxic effects of the monovalent and divalent cations can be neutralized by combining the salts in certain definite proportional concentrations.

These experiments go to show that an adequate appreciation of the fundamental causes of protoplasmic behavior can be obtained only when we can discriminate experimentally between different regions of the protoplasm of a cell.

ELASTICITY AS AN INDICATOR OF PROTOPLASMIC STRUCTURE[1]

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LIVING protoplasm, viewed through the microscope, presents the picture of a heterogeneous suspension of minute granules and larger liquid droplets. If we assume the smaller particles to be liquid in nature, as are the larger globules, then the suspension becomes an emulsion throughout. This microscopic picture may well have its counterpart in the realm of ultramicroscopic dimensions of the living substance. These facts have been the basis of the wide acceptance of the emulsion hypothesis of protoplasmic structure. This theory, expressed in different terms, was advanced by Bütschli, who characterized protoplasm and non-living jellies as "alveolar" in structure.

Bütschli postulated the alveolar structure for both protoplasm and gelatine; consequently, the hypothesis commanded the attention of chemists who, however, early discarded the conception, owing to the large size (2μ) of the alveoli. Other but inexact adverse criticism has been based on the possibility of producing the alveolar structure with fixatives. This led Hardy, Gaidukov and others to erroneously regard the alveolar structure in protoplasm as an artifact, a post-mortem process. This is not necessarily true. Typical Bütschlian alveolar protoplasm is clearly visible in the living ectosarc of numerous Protozoa.

The criticism of the chemists, that structural units 2 μ in size are altogether too large for colloidal systems, was readily overcome by imagining the size of the dispersed globules decreased, thus retaining essentially the idea of Bütschli that living and non-living jellies are emulsions.

[1] Address given at the symposium on "The Structure of Protoplasm," American Society of Naturalists, New Haven, Conn., December 30, 1925.

This hypothesis was early advanced by Wo. Ostwald who based his "emulsoid" conception of gel structure chiefly on the admittedly convincing fact that "emulsoids" of unknown structure show an increase in viscosity with increase in concentration of the dispersed phase just as do emulsions; and this is not true of "suspensoids," *i.e.*, of solid suspensions.

It is surprising how firm a hold the "emulsoid" hypothesis of the structure of organic jellies obtained on many chemists and even more so on biologists—among which latter it is still held by some—in view of the fact that, first, there are several fundamental physical properties of jellies which are not possessed by emulsions, and second, the theory never was accepted by the majority of leading colloid chemists, among whom are Donnan, Duclaux and Zsigmondy. (Freundlich adopted the terminology, which he has now rejected, without fully accepting all that the terms connote.) Duclaux has gone unnecessarily far in denying the liquid-liquid structure of gels by altogether excluding suspensions, both solid and liquid, from colloids. He says, "Les propriétés des colloides sont différentes de celles des suspensions, et les travaux faits sur ces dernières n'éclairent en aucune manière la nature des premiers. Aussi n'en sera-t-il plus question dans ce qui suit." While it seems unnecessary to take so drastic a step as to exclude such classical colloids as the metal sols from colloids in general, yet Duclaux expresses the consensus of opinion when he emphasizes that the two main groups of colloids, the lyophobes and the lyophiles, are fundamentally different types of systems.

The usual difficulty of defining protoplasm arises in connection with such a problem as the present one. That living matter contains non-living material, such as salts and organic food, is evident. To exclude all protoplasmic inclusions would mean, of course, the death of the living substance in time. Therefore, we may, if we wish, regard an oil globule in protoplasm as living in so far as it belongs to a living system. This does not preclude,

however, regarding the ultimate living substance as a system somewhat simpler than the heterogeneous mixture to be observed through the microscope. It is in this latter sense that protoplasm will be viewed in the present consideration of its elasticity and structure. The justification of doing this will be appreciated by a comparison with milk.

Milk consists of an emulsion of butter-fat dispersed in an aqueous medium of casein and other substances. To remove the fat droplets from milk means that we no longer have milk, but if we are interested in one of the properties of milk such as coagulation, and are in search of the ultimate structure which is the seat of this behavior phenomenon, then we must look beyond the visible emulsion of butter-fat. It is the casein in milk which coagulates. The fat globules play no active part in this process.

A more inclusive interpretation than the one we are necessarily restricted to here in a consideration of the elasticity of protoplasm will close this article.

An ingenious theoretical attack on the question of gel structure is the mathematical analysis by Hatschek.[2] He tentatively assumes gelatin to be an emulsion the dispersed liquid globules of which are taken to be rhombic dodecahedrons, or, for simplicity's sake in calculation, cubes. If these cubes, whose edges are regarded as of unit length, are stretched or compressed to the length L, the section remaining square, the sides of the base of the rectangular prism so produced will be $\frac{1}{\sqrt{L}}$. The total surface will then be $\frac{2}{L} + 4\sqrt{L}$, and as the original surface is 6, the increase in surface S will be

$$S = \frac{2}{L} + 4\sqrt{L} - 6.$$

The only energy resident in a liquid-liquid system which can be responsible for the presence of an elastic

[2] Trans. Faraday Soc., 12, 17–22, 1917.

property is the interfacial tension between the two liquid phases. This energy is dependent upon degree of dispersity and order of magnitude of the interfacial tension; consequently, the increase in surface S, multiplied by a constant K expressing the two energy factors just mentioned, must be equal to the work done in producing elongation, *i.e.*, the stress W is $\int$ WdL, and we have, therefore,

$$WdL = KdS.$$

Differentiation of the first equation (for $K = 1$) gives

$$W = \frac{2}{\sqrt{L}} - \frac{2}{L^2}$$

If the equation $WdL = KdS$ is plotted with values of W as ordinates and of L as abscissae, we obtain positive values of $W<1$ for all values of $L>1$, with a drop in the values of W beyond $L = 2.5198$, at which point the curve has a downward inflection. We thus have the striking result that the stress increases until the structure has been stretched two and a half times its original length and then decreases; an impossible situation, in contradiction to all experience with elastic bodies.

Hatschek further compares that portion of the curve $WdL = KdS$ in which $L<2$, with experimental curves (by Bjerkens) of elongations of gelatine and rubber up to 100 per cent. ($L = 2$). The theoretical and experimental curves fail to coincide and differ strikingly in general character.

On the basis of the above mathematical analysis Hatschek concludes that "the theory that gels consist of two liquid phases must be pronounced untenable."

It is difficult to visualize the mechanism which could be involved in an elastic system whose structural units are spherical, whether liquid or solid, but if the units of structure are of linear configuration, long tenuous fibers which interlace, then the system is quite evidently an elastic one. Such a structure has now been widely accepted as the most likely one of elastic gels, and has been graphically termed a "brush heap." Whether the fibers

are chain molecules, as they may be in gelatine, or aggregates of molecules in the form of slender crystalline fibers of colloidal size, as they possibly are in soap jellies, or of microscopic dimensions, as exist in soap curds, is still in most cases an unsettled question. That the size of the structural units and their arrangement differ in different gels is undoubtedly true.

The presence of elasticity in certain systems of known fibrous structure (soaps), and the absence of an elastic property in systems of known spherite structure (emulsions and some inelastic soaps), both support the hypothesis of an intertwining mass of fibers as the structure of elastic jellies. An interesting bit of evidence which convincingly demonstrates the absence of elasticity in emulsions is that reported by Kelly.[3]

The ovoid globules which constitute the dispersed phase of latex from *Hevea,* the source of commercial rubber, possess an external layer of protein enclosing a droplet of hydrocarbon with fatty acids and other minor rubber constituents. On coagulation of the latex the globules come into contact and adhere. The protein membranes which hold the globules of the coagulum in mutual contact give to rubber its elasticity. It is customary in rubber manufacture to mill the crude product. Milling causes a breaking down of many globules and a consequent release of the enclosed hydrocarbon oil. Decreased elasticity results from increased milling. The explanation is evident. In unmilled rubber the oily content of the globules has no effect on the system since the hydrocarbon is fully surrounded by the elastic protein gel. With increased milling there is an increase in released hydrocarbon oil. An emulsion results, and the greater the milling the greater is the amount of emulsion in proportion to protein jelly, and the less is the elasticity of the system.

That solid spherical particles are no more conducive to elasticity than are liquid ones is shown by some recent

[3] Monograph of the Third National Colloid Symposium, 1925.

measurements of the elastic values of dilute colloidal solutions.

Freundlich and Seifriz[4] developed a technique for microscopically measuring the stretching capacity of thin solutions. The method consists in suspending, with the aid of microneedles mechanically manipulated, a minute (7 μ) nickel particle in the solution under observation, then attracting this particle with a special electromagnet, and measuring with an ocular micrometer the distance the particle returns, as it will do if the solution is elastic; in glycerine, for example, there is no return whatever. It was found that a sodium soap (Na-stearate), vanadium pentoxide (V_2O_5), and the dye benzopurpurin, are elastic, and that iron oxide (Fe_2O_3) is inelastic. The first three elastic solutions possess rod-shaped colloidal particles, the last inelastic one has spherical particles. Further, the elastic V_2O_5 and benzopurpurin solutions are doubly refractive, the Fe_2O_3 is not.

In some experiments of Freundlich and Schalek[5] it was found that a Na-stearate soap from Kahlbaum deviated from Poiseuille's law when viscosity measurements were made with a Hess capillary viscometer, as is to be expected of an elastic solution, while Na-stearate from Merck showed no such deviation. Seifriz[6] investigated these soaps, and also Na-oleate, both as to their elastic properties and the shape of their microscopic particles.

Na-stearate—Kahlbaum is highly elastic and has rod-shaped microscopic particles. Na-stearate—Merck is inelastic (which explains the fact that it follows Poiseuille's law), and possesses spherites, not fibers, as dispersed particles: this is also true of the inelastic Na-oleate soap.

It is quite clear from all the above diverse data that elastic colloidal solutions are of fibrous structure.

There is no property of protoplasm so characteristic and ever present as is its elasticity. The micromagnetic method has been applied to the living substance, and the

[4] *Zeitschr. physik. Chemie, 104,* 233–261, 1923.

[5] *Zeitschr. physik. Chemie, 108,* 153–174, 1923.

[6] Monograph of the Third National Colloid Symposium, 1925.

elasticity of protoplasm demonstrated and measured. But there are many other pieces of evidence of the high elastic value of protoplasm. Strands of protoplasm may be stretched to extraordinary lengths with the aid of micro-needles. The nucleus of the red blood corpuscles of *Cryptobranchus* may be stretched to twenty times its original diameter and on being released quickly returns to nearly its original size. The remarkable protoplasmic processes formed on unripe Echinoderm eggs ("oocyte papillae") may be bent through 90° and yet return to the upright position with a suddenness which only can be due to marked rigidity. Conklin[7] has recently demonstrated elastic qualities in the protoplasm of dividing *Crepidula* eggs—the centrosomes and other cell structures show marked elasticity. Extraordinarily tough and elastic are some cell membranes, such as the pellicle of the human erythrocyte.

The high elastic properties of protoplasm not only point to a fibrous structure but also to the gel state. Bayliss and others are wont to emphasize the sol nature of protoplasm. It makes little difference what we call protoplasm, whether sol or gel, so long as we remember that its physical properties are the properties of jellies. If the ability to flow is our criterion of the sol state then protoplasm is usually, but by no means always, a sol; but there are other indicators of the colloidal state such as elasticity, rigidity and imbibition, and these are gel characteristics. It is of interest in this connection to note that an elastic soap solution of only 0.1 per cent. concentration and with a viscosity value less than twice that of water—to the unaided eye no more viscous than water—possesses a readily measurable elastic value.[4]

While it is now generally agreed that the structural unit of elastic solutions is linear in configuration, the magnitude of the unit in each case is not known. The work of Procter, Wilson, Loeb and especially some recent work of Langmuir has led these workers to regard the

[7] *Jour. Exp. Zool.*, *22*, 312–373, 1917.

[4] *L.c.*

molecule as the unit of structure in gelatine. The dimensions of these chain molecules have actually been estimated by Langmuir. In other elastic gels it seems quite probable that the linear molecules form aggregations which become slender tenuous crystalline fibers of colloidal size, reaching in the case of soap curds microscopic proportions.

In harmony with the fibrous structure here postulated for protoplasm is the fact that all organic solutions which are found in living cells, those which are foods, such as the sugars, starches and fats, but especially those which are essential constituents of protoplasm, the proteins, all have linear molecules made up of open chains of atoms: none shows the cyclic structure.

If we grant—and it seems that we must—that the fundamental structure of protoplasm, the structure of the ultimate living constituent of protoplasm, is fibrous—made up of either a homogenous orderly distribution of chain molecules, or of an entangled mass of intertwining crystalline fibers—then where in this structural plan are the foods to be found, especially the fatty substances, which in the microscopic picture of protoplasm occur as an emulsion? A possible and evident answer to this question is the following one.

The emulsion of the non-living food constituents of protoplasm, in so far as this emulsion is ultramicroscopic, must exist in the interstitial spaces of the fibrous structure of the proteinaceous ground-substance; in the intermolecular spaces if the structural units are molecules, and in the intermicellar spaces if the units are colloidal. Such a picture of protoplasm—an essentially non-living suspension of food particles enmeshed in the fibrous structure of the living protein matter—aids toward an interpretation of some physiological problems. One such is the controverted question of the nature of the mechanism of the entrance of salts on the one hand, and the ready passage of fat soluble substances on the other, into the cell. Overton's lipoid membrane was a successful attempt to explain the latter fact. He has

since been repeatedly and often unjustly criticized for neglecting the necessary entrance of water soluble salts. This he did not do; on the contrary, he recognized that a lipoid membrane satisfies only the experimental phenomena which he observed, but leaves unexplained how salts enter the cell. The advocates of the protein membrane have, on their side, been unable to account for the very rapid entrance of fat-dissolving substances such as alcohol, ether and chloroform.

The outer layer of protoplasm has a morphological identity distinct from the interior mass; there is much evidence to support this. There is also evidence pointing toward a different constitution of the membrane from that of the interior protoplasm, but the difference is undoubtedly one of degree, of relative proportions of chemical constituents, or of compactness of arrangement. The high elasticity and rigidity of the membrane suggest the latter. Certainly the membrane is fundamentally the same type of colloid-physical system as is the protoplasmic mass as a whole. Through such a system, of interlacing proteinaceous fibers permeated by a fatty emulsion, both water-soluble salts and fat-dissolving substances will have their respective avenues of passage into the living protoplast.

We can, therefore, regard protoplasm as essentially a protein-like substance, fibrous in structure, which is permeated by an emulsion of food material. This emulsion, in an ultramicroscopic state, may play a prominent rôle in some physiological processes, but those physical properties which so fundamentally characterize protoplasm, such as elasticity, rigidity and imbibition, exist only in virtue of the fibrous structure of protoplasm.

THE STRUCTURE OF PROTOPLASM IN AMOEBA[1]

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THE following refers to Amoeba of the proteus type. This form is well adapted for observations on the structure of substance in the living state. It can readily be flattened under the cover-glass until it is thin enough so that by changing the focus all parts can be clearly seen under the highest magnification, both in light and dark-field illumination, and while it is thus flattened the processes of locomotion, digestion and various other phenomena continue, making it possible to observe them closely. Moreover, if a cover-glass of the proper thickness is used, the pressure on the amoeba can be changed by lowering and raising the objective and the effect observed in detail throughout the whole process of changing the pressure.

In amoebae of this form there is an outer elastic layer, the plasmalemma, about .25 micron thick; and immediately below there is a hyaline layer which varies greatly in thickness in different regions of the body and in any given region at different times. It may be so thin that it is practically absent or so thick that it appears like a blister. It frequently contains scattered granules which are in Brownian movement, practically as violent as it is in similar granules suspended in the surrounding culture medium, showing that the consistency of this layer is similar to that of water.

The rest of the body, constituting by far the greater portion, consists of a granular substance which, when the amoeba is at rest, appears to be precisely the same in structure throughout. But when it is in motion it can be very readily seen that there is an elongated central por-

[1] Address given at the symposium on "The Structure of Protoplasm," American Society of Naturalists, New Haven, Conn., December 30, 1925.

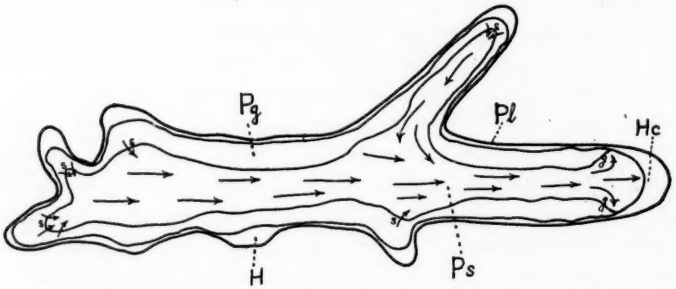

FIG. 1. Sketch of Amoeba of the proteus type as seen from above. Pl, plasmalemma; H, fluid hyaline layer; Hc, fluid hyaline cap; Pg, plasmagel; Ps, plasmasol; Arrows, direction of flow; s, regions where plasmagel solates and becomes plasmasol; g, regions where plasmosol gelates and becomes plasmagel.

Note that the plasmagel is very thin under the hyaline cap. The fluid portion freely passes through it here, but the granules and the vacuoles do not, indicating that it has very small pores.

tion which is definitely differentiated from a layer surrounding it. The central portion I have designated the plasmasol and the outer portion the plasmagel (Fig. 1).

The following structures are found fairly uniformly distributed throughout both of these portions of the amoeba: food-vacuoles, a contractile vacuole, a nucleus, spherical granules about .25 micron in diameter, angular granules about 1 micron in diameter, spherical bodies, varying greatly in size, and various crystals, each in a vacuole. The food-vacuoles are surrounded by plasmalemma taken in with the food in the process of feeding. They contain food in various stages of digestion. The spherical bodies are partially elaborated food. They are formed in the food-vacuoles and are set free by the disintegration of the plasmalemma which surrounds the vacuoles. Normally the crystals appear to be suspended free in the cytoplasm, but if neutral red is added to the culture medium it can readily be seen that each crystal is suspended in a vacuole. The contents of these vacuoles becomes brownish yellow in neutral red, indicating alkalinity and differentiating them from the surrounding cytoplasm which does not stain.

When the amoeba is at rest all these structures are, with the exception of Brownian movements, practically at rest, those in the plasmasol as well as those in the plasmagel; but when it is in motion, the plasmasol continuously flows forward and as it flows forward the vacuoles and granules in it freely tumble over each other apparently precisely like particles in suspension in a stream of water, while those in the plasmagel retain a fixed spacial interrelationship. This results in a very definite perceptual differentiation between these two structures, and it shows that they differ radically in consistency, the plasmagel being relatively solid and the plasmasol relatively fluid. Furthermore, if the plasmagel breaks, as it frequently does, at the distal end of advancing pseudopods, especially in temperature around 30°, the plasmasol spreads out very freely in the watery substance of the hyaline layer under the plasmalemma. These and other similar observations demonstrate conclusively that the plasmasol has, at least sometimes, the properties of a fluid not very much more viscous than water.

The crystals in the plasmasol are, as previously stated, in vacuoles. These vacuoles contain a substance surrounding the crystals which varies greatly in consistency. It is sometimes so viscous that there are no perceptual Brownian movement in the crystals, and at others so fluid that the crystals move about in the vacuoles apparently as freely as they do in water, now striking this wall and then that. The food-bodies are also in vacuoles containing a fluid (Fig. 2).

Whether or not the granules and the spherical bodies are in vacuoles could not be ascertained. However, since the crystal-vacuoles and the food-vacuoles contain substances having fluid properties and since these vacuoles are suspended in a fluid, it is evident that the plasmasol is an emulsion.

In the plasmagel the various granules and vacuoles, as previously stated, continuously retain their relative positions during locomotion as well as during rest, indi-

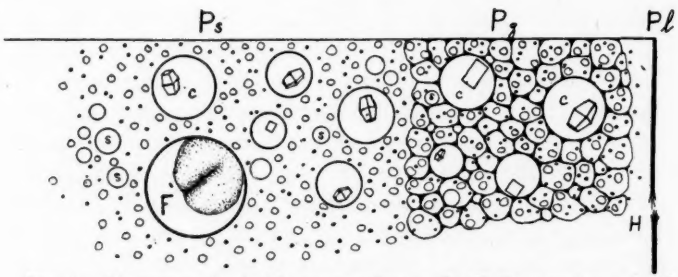

FIG. 2. Diagrammatic sketch representing in detail the structure of different parts of Amoeba. Pl, plasmalemma, torn near one end (note the shreds indicating a fibrous structure); H, hyaline layer; Pg, plasmagel (the framework represented could not be seen, but the restriction observed in the Brownian movement of the granules indicates vacuoles consisting of fluid surrounded by a more solid substance); Ps, plasmasol; F, food-vacuoles (containing a mass of food material and a considerable quantity of liquid surrounded by a sheet of plasmalemma); c, crystal-vacuoles; s, spherical bodies; dots and angular outlines, granules.

cating that it has a fairly rigid structure. Moreover, if the plasmagel is distorted by pressure, it immediately resumes its original form, showing clearly that it is elastic. All the granules in it are usually, however, in marked Brownian movement, and frequently also the crystals, indicating that they are suspended in a substance having fluid properties; and these Brownian movements are at times nearly as violent as they are in similar granules suspended in the culture medium, showing that the consistency of the substance in which they are suspended is similar to that of water. But if this is true, how can the rigidity and the elasticity of the plasmagel be explained?

I have demonstrated that while the crystals are frequently in marked Brownian movement, these movements are definitely confined to the limits of the space enclosed by the vacuoles surrounding them. Detailed observations on the Brownian movements of the granules in the plasmagel indicate that they also are similarly restricted. If this is true, it is obvious that the fluid substance in which they are suspended is enclosed by a more solid substance; that is, that the plasmagel is divided into

innumerable minute compartments separated from each other by layers so interrelated as to form a framework and that the rigidity and the elasticity observed in the plasmagel is due to this relatively solid framework (Fig. 2).

It would thus appear that in Amoeba we have in the plasmagel a typical Bütschlian alveolar structure and in the plasmasol a typical emulsion;[1] the one essentially solid, the other essentially liquid. But it should be emphasized that this difference in structure can not, at least in reference to some vital processes, be very significant, for during locomotion the plasmasol changes continuously into plasmagel and *vice versa,* as indicated in the following paragraph.

As the plasmasol in its forward flow reaches the anterior end and there comes in contact with the plasmagel the granules and vacuoles in it can be seen to become fixed in their relative positions, indicating that the plasmasol here gelates and becomes plasmagel. In the plasmagel the granules and vacuoles hold their relative positions until in the process of locomotion they reach the posterior end. Here they can be seen to move inward, and as they proceed their relative position can be seen to change, at first slowly and then more and more rapidly until they are fairly tumbling about among each other, showing definitely that the plasmagel here solates and becomes plasmasol. Thus there is a continuous transformation of the one into the other (Fig. 1).

[1] There has been much discussion concerning the effect of fixing agents on the structure of protoplasm. Some contend that fixed and stained cells give a true picture of their structure in the living condition. Others contend that practically all the differentiated structures observed in such cells are due to the action of the reagents used in fixing and staining. The plasmasol in fixed and stained amoebae has a typical alveolar structure and appears precisely like the plasmagel. Here fixing seems to have altered the structure, for in living specimens the structure of these two portions clearly differs radically. Moreover, if specimens are observed while they are being killed one can frequently see, in thickened regions of the hyaline layer under the plasmalemma, the scattered suspended granules arrange themselves in irregular rows so interrelated as to form a network, indicating an alveolar structure. Here the structure is unquestionably modified, at least to some extent, by fixation.

The plasmalemma, as stated, is a thin elastic layer. If pressure is brought to bear on the cover-glass there is frequently formed a local thickening in the hyaline layer under it. This is apparently due to rupture of the walls of some of the alveoli in the plasmagel, permitting of exudation of the fluid and granular contents. If more pressure is exerted the plasmagel gives way and the plasmasol flows out, first only the smaller granules and vacuoles, then the larger ones. Before this has proceeded far, the plasmalemma usually breaks and the contents of the blister begins to flow out. As the granules and vacuoles pass through the opening, it can frequently be seen that, if they are somewhat larger than the opening so that the plasmalemma is stretched as they are forced through, they leave the opening with a very definite spurt, demonstrating conclusively that the plasmalemma is elastic. If the edges of the opening in it are carefully examined, fibrous shreds are usually found, indicating that it is essentially fibrous in structure (Fig. 2). The plasmalemma, then, is a thin elastic membrane having at least a fibrous substratum in its structure.

It is consequently evident that different portions in Amoeba vary greatly in structure. And now the question arises as to which portions and what substances in it are actually alive. This question in reference to various other organisms has been extensively and warmly discussed. Some contend that all the actually living substance in cells is in the form of granules, bioplasts, plasmasomes and the like, others that it is in the form of fibers and still others that it is in the form of interlacing sheets, all holding that the more fluid portions together with numerous more solid particles, the so-called inclusions, are non-essential (Altmann, Arnold, Flemming, Bütschli, Beijerinck and many others). Some hold that there are in organisms specific, extremely complex chemical compounds and that only these are alive, all others being merely accessories (Verworn, Reichert, *et al.*). Others hold that the essentials in living substance con-

sist in numerous different mixtures of chemical compounds partially isolated from each other, *i.e.*, in a polyphase system (Hofmeister, Hopkins, *et al.*). It is consequently evident that there is great diversity of opinion regarding the question under consideration. This diversity of opinion is doubtless closely associated with diversity in the views held concerning what constitutes the status of being alive and with the absence of rigid definition of these views.

Whatever else may be said in reference to this, there is fairly general consensus of opinion among biologists that being alive involves the following processes: adjustment, metabolism, growth, reproduction and response. If this is true, it is evident that only that substance in which all these processes occur is alive. In Amoeba some of these processes cease if the nucleus is removed or if a certain amount of cytoplasm is eliminated or if the plasmalemma is destroyed or if a certain amount of water is withdrawn, etc., indicating that all the processes mentioned occur only in substances so organized as to constitute a cell or a larger unit, that in accord with this view water and proteins and oxygen and salts and all the other chemical compounds known to be associated with vital processes are quite as much alive as any other compounds, no matter how complex they may be, if indeed there are any others,[2] and that the essence of being alive consists in organization rather than in specific substance.

[2] I realize full well that if protoplasm is defined as the substance necessary for vital processes, it will probably never include all the substance in any cell, for every cell probably always contains some substancess (certain solutions, crystals, granules, fibers and the like) which have no immediate function in vital processes; and also that it will logically include some substances outside the cell, for some of these are probably always necessary for all vital processes. The latter can, of course, readily be excluded by definition. But to differentiate sharply those substances in the cell which function in vital processes from those which do not seem to me, in the present state of knowledge, to be quite impossible, and it is consequently evident that protoplasm can not be sharply differentiated by means of the definition presented, but the same doubtless obtains in reference to every other definition that can be formulated.

If then protoplasm is defined as living substance, its structure must involve the cell as a whole, not the nucleus by itself or the cytoplasm or this portion or that portion or the other portion, but all these together organized into a working coordinate system; and if this obtains, the structure of protoplasm can no more adequately be described by portraying the structure of any given part, no matter how detailed it may be, than can a Ford or a Rolls-Royce by elucidating the structure of a wheel or any other individual part.

Hofmeister long ago pointed out that one of the outstanding characteristics of living systems is the fact that within a given cell there occur simultaneously, side by side, a multifarious aggregate of different processes and that this is possible only on the assumption that the cell is divided into numerous partially isolated subsystems containing different mixtures of chemical compounds, making it possible for a given process to occur in one compartment and a radically different process in an adjoining compartment. This, it seems to me, has a most profound bearing on the problems concerning the structure of protoplasm, for it apparently shows that, whatever else may obtain, the cell contents in the living state must somehow contain partially isolated subsystems or compartments.

That the number of such compartments must be extremely large is clearly shown by the fact that there are in each cell practically innumerable simultaneous processes of such a nature that the regions in which they occur must differ chemically and must be partially isolated. For example, in the hydrolysis of nucleic acid alone there have been discovered more than a dozen definite steps, each requiring a specific enzyme in a specific chemical solution, and in a single chromosome of Drosophila there are apparently scores of different groups of chemical mixtures definitely separated from each other, each of such a nature as to make possible the development of a given group of characters.

What sort of structure is necessary for such subdivision of the cell? It seems obvious that it could obtain in a Bütschlian alveolar system with a continuous solid phase and a discontinuous liquid phase, in a suspension, with a continuous liquid phase and a discontinuous solid phase consisting of granules or fibers, in an emulsion with both phases liquid, in a structure with both phases relatively solid, or in any combination of these.

In Amoeba the surface layer (plasmalemma) is definitely differentiated. It is doubtless functionally of great importance, for it probably has much to do with the regulation of the degree of isolation between the cell contents and the surrounding medium. It is, as stated, fibrous and highly elastic, and it apparently varies greatly in permeability. Its most important properties can be explained on the assumption that it consists of closely interwoven protein fibers and a lipoid substance filling the interstices. During the process of feeding, a portion of it is taken in with each food-vacuole. This is later transformed into plasmasol and new plasmalemma is formed to take its place. The plasmasol is clearly partly in the form of an emulsion and partly in the form of a suspension. There are numerous discontinuous vacuoles containing fluid and discontinuous solid crystals and granules suspended in a continuous fluid. The plasmagel is probably a typical Bütschlian alveolar structure in which there are suspended in the more or less liquid phase numerous solid crystals and granules. The one, however, as previously stated, changes freely and, during locomotion continuously, into the other and *vice versa*. The one, as a whole, has the properties of a fluid and is relatively inelastic, the other, as a whole, has the properties of a solid and is highly elastic. Either unquestionably constitutes a highly essential portion of the cell, indicating that the essentials in the structure of protoplasm consist neither of solidity nor fluidity nor elasticity, but of a subdivision into innumerable compartments, each partially chemically isolated from the other and from the sur-

rounding medium, a subdivision which may obtain in an emulsion or in a suspension, as well as in an alveolar system or a structure consisting of discrete interlaced fibers with a more fluid substance between them.

The structure of protoplasm in Amoeba and the processes observed in it support the contention of F. G. Hopkins (1913) set forth in the following words:

> We can scarcely speak at all of living matter in the cell. At any rate we can not, without gross misuse of terms, speak of the cell life as being associated with any one particular type of molecule. Its life is the expression of a particular dynamic equilibrium which obtains in a polyphasic system. Life . . . is a property of the cell as a whole, because it depends upon the organization of processes, upon the equilibrium displayed by the totality of the coexisting phases.

THE PHYSICAL STRUCTURE OF THE PROTOPLASM OF SEA-URCHIN EGGS[1]

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DOUBTLESS every biologist who has ever looked at protoplasm has wondered about its true nature. Certainly many have speculated as to the physical properties which endow it with life.

But until a few years ago no physical study of protoplasm was attempted. Cytologists studied its appearance with closer and closer detail. The various aspects of its morphology, especially after treatment with killing fixatives, were described again and again, and many names were invented for the structures found both in living and fixed cells. But the morphologist, in spite of his painstaking observations, has taught us very little concerning the physical structure of protoplasm. He has called it a viscous liquid, but how viscous it is he does not say. Nor has he been able to decide whether it is really a liquid, or a plastic solid, a gel, a network or a foam.

In recent years at least a beginning has been made in the application of physical methods of investigation to the living cell. It is concerning the results of such investigation that I propose to speak. For the sake of simplicity I shall in the main confine my remarks to the egg of the sea-urchin.

This egg has been widely used in biological studies, but even at present there seems to be some doubt as to the simple details of its morphology. Visual observation shows it to be a mass of granular protoplasm surrounded by a thin membrane which is free from granules. This outer membrane does not show very clearly, for it

[1] Contribution from the Zoological Laboratory of the University of Michigan. Read at the Symposium of the American Society of Naturalists, New Haven, December 30, 1925.

apparently has a refractive index very close to that of sea-water. Its presence can, however, be demonstrated in a variety of ways. Thus Herbst ('93) succeeded in isolating it by pressing out the interior protoplasm. It is a true membrane and has the properties of a gel, that is to say, it is more or less rigid. Evidence has been presented to show that this gelatinous membrane is the membrane which controls the osmotic phenomena of the cell (Heilbrunn '15).

This conception of the fundamental morphology of the sea-urchin egg cell agrees in general with the views of the older botanists with regard to the plant cell. Thus Naegeli and Pfeffer thought of the cell as consisting of granular protoplasm surrounded by a thin more solid layer of hyaloplasm which served as an osmotic membrane, and this view, although now almost obsolete, has survived in a book of Haberlandt's ('14). Moreover the botanists showed that when the outer membrane was torn, the naked protoplasm of the interior always formed a new membrane at its surface which resembled the original membrane (Naegeli '55, Pfeffer '77). Similar observations have also been made for animal cells (Kühne '64 for Stentor, and O. and R. Hertwig '87 for the sea-urchin egg). These observations are to my mind incontrovertible evidence that the membranes at the surface of cells in general, and of the sea-urchin egg in particular, are precipitation membranes of the Traube type.

So far no one has attempted to discover the nature of the precipitation process which occurs at the surface of momentarily naked protoplasm. This is an extremely important problem and one readily open to attack. It is easy to observe the precipitation under various chemical and physical conditions. During the past summer a few crude preliminary experiments were made in the case of the sea-urchin egg. As far as they have gone they indicate that the formation of a surface membrane in this cell is favored by the presence of calcium salts and hindered or prevented in their absence.

Apparently the membrane of the sea-urchin egg resembles the investing membranes of other cells in bearing a negative electric charge at its outer surface. Evidence in support of this view has been presented in earlier papers ('23, '25).

In the cell we are considering, only the thin outer membrane is rigid. The interior is highly fluid. The absolute viscosity of the interior protoplasm of the sea-urchin egg may be measured by a method which involves no injury to the cell. When the eggs are centrifuged it is easy to determine the speed of movement of the granules they contain. Both the speed of the smaller granules or microsomes[2] and that of the larger pigment granules can be observed. These speeds can be used as a measure of the fluidity or viscosity, provided that the specific gravity of the granules as compared to the specific gravity of the medium surrounding them can be estimated. The specific gravity of the granules can be approximately determined by centrifuging them outside the egg in solutions of known specific gravity. It is possible to calculate the specific gravity of the material surrounding the granules from the specific gravity of the egg and the specific gravity of the granules. Knowing these quantities, it is possible to apply Stokes's formula for the movement of a spherical particle through a fluid and thus arrive at the viscosity. But it is essential to use a corrected form of the Stokes formula, for as originally derived it applies only to the movement of a single particle.

There is no need to discuss further details of these measurements, as they are being presented elsewhere (Heilbrunn '26b). The results are striking. The viscosity of the protoplasmic medium in which the granules are suspended is approximately only twice that of water. This is at first surprising when we consider that oils are hundreds of times as viscous as water and that sulphuric acid is twenty times as viscous as water. But in reality

[2] Some cytologists might prefer to call these granules macrosomes. But in the living egg they are really very small, being only about three tenths of a micron in diameter.

the value obtained is exactly what we would expect for a protein solution, and this is most certainly the type of solution with which we are concerned.

The fact that measurements reveal such a low viscosity for the fluid surrounding the granules of the sea-urchin egg is convincing proof that there can be no network of strands or other solid material in the protoplasm. Further proof, if any were needed, is given by the fact that the viscosity values obtained at different centrifugal speeds are identical (Heilbrunn '26a). If a network of strands existed between the granules, the higher centrifugal speeds would be more effective in breaking down the network, and lower viscosity values would be obtained with such speeds. The rate of movement of the granules under the influence of different magnitudes of centrifugal force is directly proportional to the force. In other words, the rate of movement is proportional to the shearing stress. Such a strict proportionality occurs only in true fluids, never in semi-solids or plastic solids (*cf.* Bingham '22).

The protoplasm of the sea-urchin egg is a suspension. It is a suspension of visible granules, which constitute perhaps a fifth of its total volume. Visibly a suspension, it must exhibit the physical behavior of a suspension. This obvious idea is one that has scarcely presented itself to the minds of biologists. Once we accept it we are immediately led to some important deductions.

In the first place, we are interested in the total viscosity of the protoplasmic suspension, that is to say, the viscosity of both granular and intergranular material. This total viscosity can be computed from the rather inexact theoretical equations of Einstein ('06, '11), and Hatschek ('10), equations which apply most nearly to suspensions in which the suspended particles are relatively far apart. Or, the total viscosity can be computed from the apparently more exact empirical formula of Bingham ('22). This latter formula requires a knowledge of the degree of concentration of granules necessary to produce zero fluidity, that is to say, a condition in which no flow is pos-

sible. It is not practicable to introduce more granules into the egg, but by shrinking the egg in various strengths of hypertonic solution we can increase the concentration of granules progressively until a condition of zero fluidity can be shown to exist by centrifugal tests. It is thus possible to apply Bingham's formula. As a matter of fact, all three formulae, those of Einstein, Hatschek and Bingham, give results which are in substantial agreement, and it is possible to show that the viscosity of the entire protoplasm is about twice, certainly not over three times, the viscosity of the fluid between the granules.

Biologists have always assumed that the intergranular material of protoplasm was extremely viscous. The demonstration of a relatively low viscosity of this hyaloplasm may make it necessary completely to revise our conceptions of protoplasmic mechanics.

In view of the fact that protoplasm is a concentrated suspension, its viscosity depends as much on the suspended particles or granules as on the suspension medium or hyaloplasm. Hence, changes in the nature of the granules may very well have an effect on the viscosity of the entire mass. Not much is known about the viscosity of concentrated suspensions and how it changes under diverse conditions. But it is obvious that with increase in concentration of suspended substance the fluidity decreases until it eventually becomes zero. It is also obvious that the ease with which the particles move over each other is a factor in determining the viscosity. This has been pointed out by Bingham ('22), who states that the friction or viscosity should be closely dependent upon the adhesion of the particles to each other. Doubtless adherent particles tend to arrange themselves in strands or other aggregations which reduce the fluidity.

All in all, there appear to be at least five ways in which the viscosity of a suspension can be altered. These are:

(1) A change in size of the suspended particles (*i.e.*, by a gain or loss of solvent from the suspension medium).

(2) A formation of new particles from the suspension medium.

(3) A change in the adhesive properties of the particles.

(4) A change in the electric properties of the particles.

(5) A change in the viscosity of the suspension medium.

The first two cases are readily understood. Both these cases involve an increase in the volume of suspended material; and this, as pointed out previously, would result in a decrease in fluidity—that is to say, an increase in viscosity. The fourth case is closely related to the third and should perhaps be included under it, for certainly a lowering of the charge on suspended particles would favor their tendency to adhere to each other. In both instances the greater this tendency for adherence the greater the viscosity. The fifth case requires no further explanation.

For the sea-urchin egg it has been possible to accumulate a considerable body of data in regard to the viscosity of the protoplasm under different conditions. This knowledge has been obtained by observing the speed of the protoplasmic granules under the influence of a centrifugal force. It might be thought that measurements of this sort would really concern the viscosity of the fluid between the granules, that is the hyaloplasm, rather than the viscosity of the entire protoplasm. That the centrifuge tests are actually a measure of the viscosity of the entire protoplasm is indicated by the fact that determinations of viscosity from speed measurements of small and large granules give similar results. As a matter of fact, even granules of a given type vary considerably in size and therefore in speed of movement when centrifuged. Hence any granule moving through the protoplasm would meet with the resistance of other granules in its path, as well as the resistance of the hyaloplasm. Moreover, any tendency of some granules to adhere to each other would produce bridges or strands of granules which would offer resistance to the movement of other granules.

It is interesting to note that the viscosity measurements on the sea-urchin egg have been supported point for point by viscosity determinations on plant cells by various botanists (for references to literature compare Heilbrunn '26c). The viscosity differences at various stages of mitosis are identical in both plant and animal tissue. So, too, the effects of temperature change and of various reagents are the same in plant cells as they are in the sea-urchin egg. There is not a single case in which the determinations on plant cells and those on marine eggs do not agree in essential details.

On the other hand, the results of the microdissectionists have now and then been in disagreement with the data obtained by viscosity measurement. But indeed the microdissectionists do not always agree with each other, even on the same sort of material under identical conditions. Thus, although Hyman ('23) agrees with the speaker in believing that dilute ether solutions cause a marked decrease in viscosity of sea-urchin egg protoplasm, Chambers ('24) in similar solutions finds an increase. The divergent results of the microdissectionists are readily understandable to any one who appreciates the extreme subjectivity of the microdissection method. Moreover, it is apparent that when the dissector judges protoplasmic viscosity by tearing cells with his needle, he is quite as much studying the formation of precipitation films at the surface of the torn cells, as he is the viscosity of their interior.

Lack of time prevents any extended discussion of the viscosity data. The effects of hypertonic and hypotonic solutions, of varying temperatures, the changes which result from treatment with a variety of inorganic and organic chemicals, have all been studied, as have the viscosity changes which occur during varying periods of physiological activity. It is to some extent possible to distinguish different types of viscosity change. Thus one can determine whether a change in the size of the protoplasmic granules is a factor, by actual measurement

of the granules either singly or in mass after extended centrifugal treatment. The formation of new granules by precipitation out of the hyaloplasm can also be detected by direct observation. The eggs are first centrifuged and are then placed in contact with the coagulating agent. In a few minutes new granules appear in what was originally the granule-free area of the centrifuged eggs. Various protein coagulants can be shown to produce such precipitations, and the increase in viscosity caused by these coagulants is easily explained.

Striking changes in the viscosity of the protoplasm of the sea-urchin egg can be obtained by increasing or decreasing the electrical charge of the granules. The fact that bivalent and trivalent cations cause a decrease in viscosity in the sea-urchin egg, and apparently also in plant cells (Scarth '24), is excellent evidence that the original charge on the granules is positive. For if the granules bore a negative charge, the greater adsorption of the bivalent and trivalent cations with their positive charges would tend to produce a neutralization of charge on the particles, and precipitation or coagulation would result. As a matter of fact, there is other evidence to show that the interiors of cells contain materials charged with a positive electric charge (*cf.* Heilbrunn '23).

The action of temperature on protoplasm is most unusual (Heilbrunn '24). For the egg of the clam Cumingia it has been shown that as the temperature rises, the viscosity first increases until, at about 15°, a maximum is reached. The viscosity then decreases as the temperature rises, until, at about 31°, it increases suddenly. Apparently the same sort of viscosity changes occur in the sea-urchin. Similar changes have also been described for slime mold protoplasm (Heilbronn '22). The presence of a maximum point in the viscosity curve at 15° is unique among colloids and indicates a special peculiarity of the protoplasmic suspension.

Another peculiarity, also apparently general for protoplasm, is the fact that low concentrations of ether and

other fat solvents cause a sharp decrease in viscosity, whereas higher concentrations cause a pronounced coagulative change, that is to say, a great increase in viscosity.

How can these peculiarities of protoplasm be explained? It seems probable that they are associated in some way with the fatty or lipoid constituents of the protoplasm. Although with considerable misgiving, it was finally decided to advance as a provisional hypothesis the idea that the typical protoplasmic granule is surrounded by a sheath or film of lipoid material. If we make this assumption we can arrive at a formal explanation of the effect of fat solvents. Let us suppose that the granules are surrounded by lipoid films. Such films would certainly be adhesive, and, as pointed out previously, the degree of adhesiveness is surely a factor which influences the viscosity. Fat solvents in dilute concentration would dissolve away part of the film. This would probably render the granules less adhesive, they would move over each other more readily and the viscosity would be decreased.[3] But with higher concentrations of fat solvents the lipoid film would be completely dissolved away, and the protein mass of the granule laid bare. It would then absorb water. As a result its volume would increase and its surface would become sticky. Both these factors would cause a rise in viscosity.

Apparently temperatures of 20° to 31° or higher act like fat solvents. A temperature of about 25° would dissolve away most of the lipoid film and cause a decrease in viscosity. Temperatures above 31° would completely dissolve the lipoid film and coagulation would result. On our hypothesis we can also explain the decrease in viscosity below 15°, for as the temperature drops we can assume that there is a gradual solidification of the lipoid film at the surface of the granules. Such a solidification would tend to decrease the friction as the granules rubbed over one another, and there would be a lowering of viscosity.

[3] Another effect of the ether is to lower the surface tension of the fatty film. This would reduce the forces holding two adjacent granules together.

The fact that distilled water produces the same effect as fat solvents indicates that the lipoid film which we have assumed to exist at the surfaces of the granules is a phosphatid rather than a fat in the strict sense of the term. For phosphatids swell and dissolve in distilled water, whereas fats do not.

It must be admitted that on the face of it our hypothesis does not appear very plausible. It was arrived at purely from a consideration of the viscosity data, and immediately an attempt was made to test it out from a microchemical standpoint. Eggs were centrifuged as completely as possible and were then treated with solutions of osmic acid. In such solutions the gray cap appeared solid black, but in addition the granular zone of the egg also showed some blackening. As far as could be determined, this was not due to the presence of an occasional fatty particle, for the dark color was evenly spread and did not appear to be in the form of scattered black dots. Search in the literature revealed the fact that the blackening of microsomes in centrifuged eggs had already been described by Lyon ('07). These observations furnish direct support of the view that the microsomes of the sea-urchin egg are surrounded by a fatty film.[4] Microchemical evidence that the pigment granules are also surrounded by a lipoid film is furnished by the very recent observation of Wilson ('25) that the outer border of these granules is colored by Janus Green in the living egg.

I believe that the observations of Runnström ('23, '24) can be interpreted as furnishing additional evidence in support of this view. Runnström describes what he believes to be a lipoid layer directly under the membrane of sea-urchin eggs. It is luminous under darkfield illumination and it disappears in the presence of lipoid solvents or distilled water. Perhaps this lipoid layer of Runnström's is to be identified with the lipoid films surround-

[4] But not absolute proof, for osmic acid is reduced by other substances as well as lipoids.

ing the granules. This interpretation is favored by the fact that the lipoid layer apparently behaves as if it were heavier than water when the egg is centrifuged.

Other facts also support the idea of a lipoid film at the surface of microsomes and pigment granules. In concentrations of lipoid solvents strong enough to produce coagulation the eggs generally "cytolyze." Such cytolysis results in a glassy appearance of the cytoplasm, which may be due to the fact that with the liquefaction of the lipoid films there is an equalization of refractive index. Moreover, cytolysis is usually followed by an escape of pigment. This pigment is soluble in water, and, inasmuch as it does not color the fatty materials of the egg, it is probably insoluble in lipoid. Perhaps it is retained in the pigment granules because of its insolubility in the surface lipoid film which is assumed to cover them.

Such indirect evidence is of doubtful value. Nevertheless, the fact that the presence of a lipoid film at the granular surface was assumed from the viscosity data and later indicated by microchemical test is strong argument for its real existence.

We are therefore led to conclude that the sea-urchin egg consists of a thin outer membrane which is a true precipitation membrane, and that within this envelope there is a fluid suspension of visible granules. The granules are of at least three varieties, lipoid granules lighter than water, microsomes and pigment granules. The microsomes and probably also the pigment granules are surrounded by a thin film, which has the physical properties of a lipoid, and more particularly those of a phosphatid. Very likely the lipoid granules are surrounded by a protein film, but so far there is no evidence in favor of this view other than the fact that ordinarily the lipoid granules do not fuse when they are centrifuged into one end of the egg. All the granules apparently bear a positive charge, and accordingly the interior materials have a charge opposite in sign from that at the surface.

It is evident that our conception of a lipoid film at the surfaces of the protoplasmic granules may have an important bearing on the theories of oxidation within the cell. Warburg's results indicate that cell oxidations occur at the interfaces between solid and fluid material of the cell, that is to say, at the surfaces of the granules (Warburg '14, '21). The presence there of lipoid material might be of great significance. Various authors have emphasized the importance of lipoids in cell oxidations. The results of Meyerhof ('23) indicate that lecithin is of especial significance in the oxidation system of the cell (*cf.* also Hopkins '25).

If it is true that the surface layer of the cell is negatively charged and that the granules of the interior are charged positively, this fact must surely be of importance in the interpretation of many cellular activities. We shall consider only a single case. In dividing eggs the granules can often be seen in the living cell to arrange themselves in lines extending from the centrospheres toward the surface. These represent lines of force, as is shown by the fact that no matter in what portion of the egg the centrospheres are thrown by the centrifuge, the granules, whether they be of one sort or another, always arrange themselves in the same manner—that is to say, in lines which radiate from the centrosphere to the surface. This observation is due originally to Lillie ('09), who experimented on Chaetopterus. He studied only fixed material, in which the phenomenon is certainly obscured. In the living Cumingia egg it is much more striking. Following centrifugal treatment, granules can be seen to be arranged in strands radiating out from the centrosphere. These granules may be either pigment granules or fat granules, depending on whether the centrosphere is thrown to the pigment zone or to the fatty zone. The arrangement of the granules is exactly what we would expect if we assumed that the region of the centrosphere bore a positive charge and that the granules were also charged positively. The granules would be repelled from the region of the centrosphere and would

arrange themselves in lines which would mark the lines of force extending from the positively charged centrospheres to the negatively charged surface of the cell.

Other observations also fit in well with our concept of the electrical charges of the cell, but there is scarcely time to discuss them here. If our view is correct it will have a profound influence on the large body of physiological knowledge concerned with the electrical currents given off by living tissue.

The scientific study of protoplasm has only begun. But we have already passed the stage in which speculation and wild surmise were the only physiological aspects of the subject. The possibility of making actual physical measurements of the viscosity of protoplasm under various test conditions has opened the door to a rational colloid chemistry of protoplasm, a colloid chemistry which will not depend on dubious and fanciful analogies, but on stern facts. The facts gathered so far indicate that the interior of living cells is, in one case at least, a fluid suspension. Furthermore, as far as they have gone, the facts favor the view that the suspended particles of this suspension are in the main protein granules surrounded by films of lipoid and possessing a positive electric charge. How long the accumulating facts will continue to support this notion, only the future can tell.

REFERENCES

Bingham.
1922. "Fluidity and Plasticity," New York.

Chambers.
1924. "The Physical Structure of Protoplasm as determined by Microdissection and Injection," in "General Cytology," edited by E. V. Cowdry, Chicago.

Einstein.
1906. *Ann. d. Physik* (4), XIX, 289.
1911. *Ib.* (4), XXXIV, 591.

Haberlandt.
1914. "Physiological Plant Anatomy" (translation of 4th German edition), London.

Hatschek.
1910. *Kolloid-Zeitschr.*, VII, 301.

Heilbronn.
1922. *Jahrb. f. wiss. Bot.*, LXI, 284.
Heilbrunn.
1915. *Biol. Bull.*, XXIX, 149.
1923. *Amer. Jour. Physiol.*, LXIV, 481.
1924. *Ib.*, LXVIII, 645.
1925. *Science*, LXI, 236.
1926a. *Jour. Exp. Zool.*, XLIII, 313.
1926b. *Ib.*, in press.
1926c. *Quarterly Review of Biology*, in preparation.
Herbst.
1893. *Biol. Centralbl.*, XIII, 14.
Hertwig, O. and R.
1887. "Untersuchungen zur Morphologie und Physiologie der Zelle," Heft 5.
Hopkins.
1925. *Biochem. Jour.*, XIX, 787.
Hyman.
1923. *Biol. Bull.*, XLV, 254.
Kühne.
1864. "Untersuchungen über das Protoplasma," Leipzig, 1864.
Lillie, F. R.
1909. *Biol. Bull.*, XVII, 101.
Lyon.
1907. *Arch. f. Entwicklungsmech.*, XXIII, 151.
Meyerhof.
1923. *Pflüger's Arch.*, CXCIX, 531.
Naegeli.
1855. Naegeli und Cramer, "Pflanzenphysiologische Untersuchungen," Zürich, Heft 1.
Pfeffer.
1877. "Osmotische Untersuchungen," Leipzig.
Runnström.
1923. "Acta Zoologica," IV, 285.
1924. *Ib.*, V, 345.
Scarth.
1924. *Quart. Jour. Exp. Physiol.*, XIV, 99, 115.
Warburg.
1914. *Pflüger's Arch.*, CLVIII, 189.
1921. *Biochem. Zeitschr.*, CXIX, 134.
Wilson.
1925. *Anat. Rec.*, XXXI, 303.

I. SURFACE FILM THEORY OF THE FUNCTION OF MITOCHONDRIA[1]

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FORTIFIED by recent advances in the science of botany, we are in a position to make more searching suggestions regarding the function of mitochondria than heretofore. These advances prove, in my opinion, that many (but not all) plant plastids, with the starch, pigment and other materials that they contain, are formed through the interaction of the mitochondria and the cytoplasm about them. If this conclusion is justified, there is reason to believe that, in accordance with Regaud's theory, the mitochondria in animal cells also act like plastids, or "eclectosomes," choosing and selecting substances from the surrounding cytoplasm, condensing them on their surface, or in their interior, and changing them into products of great diversity.

The view that plastids are of mitochondria origin was first advanced by Lewitsky ('10), and has since been supported by Alvarado, Cavers, Emberger, Forenbacher, Guilliermond, Mangenot, Maximow, Mirande, Moreau, Nassonov, Noël, Pensa, Politis, Twiss and many others.

But in our search for facts we must not be unduly influenced by the number of witnesses: too much is at stake, involving much that is fundamental in our conception of cellular physiology. There are, moreover, others who either believe that the plastids arise in a totally different manner, or who very properly demand further and more convincing evidence before committing themselves. Among these the following names may be mentioned: Choloduji, Harper, Lowschin, Lundgegardh, Mottier, Rudolph and Sapehin.

[1] Address prepared for the symposium on "Studies on the Integration of Cellular Components."

In 1919 Harper wrote as follows:

> None of the evidence so far adduced as to the specific genetic relationship between chondriosomes (mitochondria) and plant plastids is in any way adequate . . . that in certain cells the plastids can be recognized as very small cytoplasmic bodies with no starch in them was adequately established by Schimper, but that the plastid bodies necessarily and regularly arise from the chondriosomes it seems to me is by no means proved by such crude and diagrammatic figures and seriations as those thus far presented.

Let us now examine the character of the evidence, including that presented in the past five years since Harper's statement. Fortunately, several of the investigators who claim that the plastids arise from mitochondria base their conclusions solely upon the examination of living material, so that we may at the outset eliminate any complications, hypothetical or otherwise, arising from the action of fixatives and stains.

It would be difficult to find more favorable objects for study than the root heads of the pumpkin, which have been investigated by Maximow. In these, spherical, highly refractile "microsomes," typical mitochondria and typical plastids may be clearly observed and followed from place to place in the streaming cytoplasm. Moreover, a complete series of intermediate forms may likewise be seen between the mitochondria and plastids. That we are faced by a process of differentiation whereby the mitochondria, with the assistance of the cytoplasm, change into the larger and structurally more complex plastids is highly probable. Yet it has been questioned on the supposition that the bodies referred to as mitochondria may, in reality, comprise, in addition to true mitochondria, other fundamentally different structures which constitute the actual primordia or anlagen from which the definitive plastids are formed. It is difficult to rationalize this attitude of mind in respect to Maximow's observations: it becomes less and less justifiable as the years go by, and investigators become more and more proficient in the identification of mitochondria. Moreover, criteria used for their recognition in other lines of work are there accepted without hesitancy and rightly so.

There is still other evidence to be considered in support of the mitochondrial origin of plastids. In the leaf buds of *Tradescantia,* investigated by Meves, it is possible to secure a very complete seriation of stages by simply passing in the same preparation from the younger to the older cells. That the mitochondria and plastids are related genetically seems clear. Another case, selected from the large number which are available, is that of *Mnium cuspidatum.* In this plant Alvarado has established a sequence of transitional forms between mitochondria and plastids which is very convincing. The young cells contain typical mitochondria and no plastids, the older cells both mitochondria and plastids. To claim again that there are other bodies, which constitute the true anlagen of the plastids, occurring side by side with the mitochondria in the younger cells, without presenting any direct evidence of their existence, would be of doubtful wisdom. Indeed, in *Asparagus officinalis* Lewitsky found that *all* the rods and granules in the young, formative cells not only behave and stain like mitochondria, but are, like mitochondria, destroyed by acetic acid and by alcohol.

In proportion as the mitochondria change into plastids, they increase in size, lose this susceptibility to the solvent action of acetic acid and gradually heap up within their interior both chlorophyll and starch.

In animal cells the mitochondria are occasionally colored naturally in the same way by pigment, but the process is more difficult to observe in the living state, because the cells are often smaller. Worthy of note, however, is the elaboration within the mitochondria of liver cells of easily detectable crystals of hemoglobin (Policard) and of protein (Noël). A close spatial relationship between mitochondria and the elaboration of more than a hundred other cytoplasmic materials might be mentioned, but their recitation would only weary you and the evidence is often much less direct.

Many investigators assume an attitude of ultra-conservatism in regard to both chromosomes and mitochon-

dria. But it seems equally safe to accept the available evidence that the chromosomes carry *some* hereditary characters and that *some* mitochondria act as accumulators in the sense outlined above. Further, that in respect to the action of mitochondria a definite series of events may be recognized, which, for purposes of discussion, may be arranged in sequence although they overlap and may take place to some extent synchronously.

First, a process of the close approximation of the molecules of certain solutes at the mitochondria-cytoplasmic interface. That this in all probability actually takes place, although it very naturally escapes our power of direct microscopic vision, seems to follow from the evidence at hand that the mitochondria are chiefly composed of variable amounts of protein and lipoid. Their staining reactions and probably their characteristic morphology depend upon this lipoid fraction. Now lipoids decrease surface tension, so that in terms of the second law of energetics we would expect approximation to occur at this interface.

Second, an act of incorporation of the material thus approximated into the substance of the mitochondria, because the materials observed within them are, in many cases, obviously of exogenous origin in relation to them. Thus, starch and compounds containing masked iron may easily be identified microchemically and are not at the beginning of the process resident within the mitochondria. That this incorporation takes place is a fact verifiable by direct observation, but to explain how it is effected is another matter. One would expect the molecules thus concentrated at the interface to remain so rather than to penetrate past the interface into a material in which at the beginning they were absent. Evidently we must hypothecate some chemical or physical change or changes in the concentrated molecules which facilitate their passage into the less fluid phase, that is to say, into the substance of the mitochondria. Another possibility would be that the mitochondria are themselves altered by the aggregation of molecules on their surfaces

and that in virtue of this alteration the molecules pass into their interior. Perhaps there are changes in both which favor this incorporation.

Third, a process of concentration in the mitochondria of the materials in question. This is evidenced by progressive increase in intensity of pigmentation, or of the amount of starch or fat, as the case may be, with resultant enlargement of the mitochondria.

Fourth, a series of chemical or physical inter-reactions between the mitochondrial material and the incoming substances, the nature of which remains very obscure, but which undoubtedly result in the building up of substances of widely different character. Parallel with each step a change occurs in the mitochondria themselves which is manifested by increased resistance to the solvent action of acetic acid and by alterations in their size and shape and staining properties.

We do not incline to Regaud's suggestion that the mitochondria may be compared in their action to the hypothetical side-chain of Ehrlich, which, indeed, he might not be equally ready to advance to-day, but it does seem that the mitochondrial-cytoplasmic surface film or interface is probably one of real importance in cellular physiology. Let us briefly consider some of its properties, by stating in slightly different terms information already well established:

(1) The film occurs in almost all living and active cells and may thus be regarded as indispensable to vital phenomena.

(2) Since the mitochondria are packed in the cytoplasm so closely, the combined surface which they offer is probably greater than that of the nuclear or cytoplasmic membranes.

(3) The aggregate position of film varies under influences which it has thus far been impossible to ascertain. As instances may be cited the localized concentrations of mitochondria in certain parts of the cell in different stages of development and in different physiological con-

ditions, also the voyaging of mitochondria from the neighborhood of the nucleus to the periphery of the cell and *vice versa*—a phenomenon repeatedly reported in certain cells.

(4) In consequence of the existence of variations in the proteolipoid constitution of mitochondria (which Regaud fully recognized) it follows that the physical properties of the film also vary. This may in part explain its apparently different action in adsorption in different cells and presumably also in catalysis.

(5) Not only does the composition of the film vary, but also the thickness of the mitochondrial material. One of the most striking properties of mitochondria, which has, I believe, been insufficiently emphasized thus far, is the constancy of their diameter in all cells of the same type, while their length is subject to great variation. If, on the contrary, cells of different kinds are compared, the diameters of the mitochondria will often be found to be quite different. It may be a matter of surface tension. Certainly these differences in diameter are indicators of protoplasmic conditions for which we have at present no other clews, the existence of which we would not expect were it not for the mitochondria.

(6) Chemical and physical action at this mitochondria-cytoplasmic film is not a one-sided process. It depends equally upon the properties of the cytoplasm, which, we know, are subject to such a range of variations that the processes there taking place may differ even in the absence of differences in the mitochondria themselves.

(7) This film is one of the most labile and easily modified structural elements in the cell. In response to injury the filaments break up into granules which often swell. In other words, there is a release of the wholly unknown forces which normally maintain the thickness of the film. Another early change in pathological conditions is manifested by a clumping together, or agglutination, of mitochondria. In this way the extent of the mitochondria-cytoplasmic film is greatly reduced. It is remarkable

that under normal conditions the mitochondria should always remain isolated from their fellows by cytoplasmic material, although in consistency they are very fluid and their motility is unrestricted so that one would instead expect them to fuse together in terms of the law of least surfaces.

If then, as we may suppose, this film is an important location, where processes of elaboration take place, beginning with electrical or some other form of adsorption and ending with the building up within the mitochondria of these substances, which we may easily recognize, what happens in the majority of animal cells in which the mitochondria retain their characteristic diameter unmodified and in which no signs of the elaboration of substances are observed? In these cases, are they acting in the same way in respect to chemical substances which do not bring about a change in their size, and which are not detectable microscopically? That they are seems highly probable.

Consider, for a moment, the adsorption of a dye like janus green, which is only slightly toxic. In the still living cells this stain will color the mitochondria specifically in a dilution as high as 1:500,000. This means that the dye, coming in contact with the mitochondria, is concentrated by them to a point which permits of visibility in a film about 0.5 micron in thickness, that is to say, we are dealing with a concentration of several thousand times. But there is little, if any, resultant enlargement in the mitochondria which take it up. Janus green is an example of a foreign substance conveniently colored. Obviously other uncolored materials may conceivably behave in the same way under living conditions.

The widespread occurrence of mitochondria in almost all kinds of cells, already alluded to, has led us to suppose that they take part in some activity common to all living protoplasm, but the significance of this fact is offset to some extent by the variations within certain limits which they exhibit in their composition and which, undoubtedly, occur likewise in the surrounding medium in

which they are suspended. It has, nevertheless, been suggested that these finely divided strands of relatively water-insoluble material may function in a single activity like protoplasmic respiration, but, unhappily, this has in no instance been proved.

In common with some other cytoplasmic materials, mitochondria are dissolved by narcotics such as alcohol, chloroform and ether, which also retard oxidative processes. When protoplasmic structure, including the widely dispersed mitochondria-cytoplasmic film, is mechanically destroyed, oxidative processes are proportionately reduced, but the effect of the mitochondrial change can not be dissociated from the others. The probability is that their function in the cellular economy is multiple, differing, perhaps, even in successive stages of cytomorphosis and between cells of different category. We do not suppose that other perhaps analogous surfaces of separation possess a constant and invariable function. Our information points, indeed, in the opposite direction.

The limitations of this problem for the cytologist are clear. If he pursues it further, he will get out of his depth, if he has not already done so. But it has been carried to the point where further responsibility may be shared by the biochemist. The latter, very properly, bases his theories upon the recognition of a fundamental heterogeneity in the structure of protoplasm, which alone makes vital activities possible, and speaks of "a polyphasic system" and of "ultramicroscopic reaction chambers bounded by reversible semipermeable membrane," and so on. We freely admit that these hypothetical, ultramicroscopic interfaces are of an order of importance second to none. We remember that areas of the cytoplasm may be cleared of all detectable structural elements, including the mitochondria, by centrifuging at high speed, without completely inhibiting their vital manifestations, and that portions of the pseudopod of an amoeba, for instance, which are structureless in appearance, are endowed with a measure of independent vital-

ity. The cell is such a tiny unit that to ascribe specific, much less exclusive functions to any element in it (with the probable but not proved exception of the chromosomes) is a risky procedure.

In conclusion, it may be said that although our ideas concerning the mitochondria-cytoplasmic film are so ill-defined, we hope, in the interests of cytology, that the biochemist will consider its possibilities equally with the better-known membranes, at least theoretically. But we must not blind ourselves to the fact that in practice this cooperation may not be forthcoming. The biochemist, in the study of permeability, will naturally choose membranes like the protoplasmic surface film, which are more readily susceptible of experimental procedures. Thus, Osterhout has selected as material the largest available cells in which the factors involved in permeability may be determined quantitatively and with precision. It may be many years before the mitochondria-cytoplasmic film can be profitably studied from the physico-chemical point of view; but, like the nuclear membrane, its importance is unquestionable.

THE INHERITANCE OF SALMON-EYE IN GUINEA-PIGS[1]

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IN November, 1919, there was obtained from "The Lintners," of Kirkwood, Missouri, a female guinea-pig which they described in an advertisement as a "red-eyed orange." They stated in a circular that the first animals of this description appeared in their colony in December, 1917, from parents of no particular breeding.

The animal obtained had the appearance of a pink-eyed red and was assumed to be such. She was mated to a male known to be homozygous for the pink-eyed factor (*p*). One half of her offspring were dark-eyed and the other half pink-eyed. On closer examination it was found that her eyes, instead of being completely pink (and therefore devoid of pigment), actually had a small amount of dark pigment surrounding the pupil. In order not to confuse this eye color with others, such as the red-eye described by Castle (1914), it was decided to call it "salmon-eye" (*sm*).

It has since been determined that there is considerable variation in the amount of dark pigment surrounding the pupil. Some animals have a comparatively large amount of pigment, which however never completely fills the iris and therefore leaves an outer ring of clear pink. The pigment itself is generally not solid in appearance, but instead is more or less granular. Other salmon-eyed animals have been found which, so far as could be determined by the naked eye, had no pigment in the iris whatever and at least with respect to the eyes resembled

[1] Contribution No. 79 from the Department of Animal Husbandry.

pink-eyed animals. In a number of instances salmon-eyed animals have been produced with eyes entirely devoid of pigment at birth but with a small amount of pigment developing when they were approximately one month old.

The salmon-eye gene also differs from the pink-eye in the effect produced on the black or chocolate pigment in the hairs of the coat. The pink-eye gene greatly dilutes black or chocolate pigment, but has no appreciable effect on red pigment. The salmon-eye gene, on the other hand, has no appreciable diluting effect on any of these pigments. It is for this reason that we have been able to demonstrate that salmon-eyed individuals occasionally are produced which do not have any pigment in the eyes and therefore in this respect resemble pink-eyed individuals. However, if these animals carry the intensity factor, *C*, and either of the extension factors, *E* or e_p, their coats are intense black or chocolate, whereas, if they actually had been genotypically pink-eyed (*p*) the black or chocolate would have been very much diluted. Additional proof will be given later demonstrating that the pink-eyed individuals with intense black or chocolate hairs are genotypically salmon-eyed (*sm*) and not pink-eyed (*p*).

As previously stated, the original salmon-eyed red female when mated to a pink-eyed red male produced equal numbers of dark-eyed and pink-eyed offspring. The results obtained can best be explained by assuming that salmon-eye, like pink-eye, is due to a recessive factor (*sm*), and that in order to produce a dark-eye the dominant allelomorphs of both factors (*P* and *Sm*) have to be present. Subsequent experimental results have verified this hypothesis. It has also been demonstrated that the pink-eye factor is epistatic to the salmon-eye.

For the above reasons the genetic composition of homozygous animals with the various eye colors mentioned should be represented as follows:

Dark-eye	*PP Sm Sm*
Salmon-eye	*PP sm sm*
Pink-eye	*pp Sm Sm* or *pp sm sm*

The original cross therefore would be represented in the following manner:

Salmon-eye (*Pp sm sm*) × *pink-eye* (*pp Sm Sm*) = dark-eye (*Pp Sm sm*) and pink-eye (*pp Sm sm*)

It seems advisable at this time to call attention to the fact that, so far as we know, there is no factor in any of the other rodents that is analogous either genetically or phenotypically to the salmon-eye factor. Many of the genes affecting eye-color in rodents belong to the *C* series of allelomorphs, but we have produced salmon-eyed animals that carried either, *C, Cd* or *Cr,* and in addition have obtained dark-eyed offspring when a salmon-eyed individual was mated to an albino (*Ca*), thereby demonstrating fully that the salmon-eye gene does not belong to the *C* series.

A number of matings have been made to test the relations of the salmon-eye gene to its allelomorph and to pink-eye (*p*) and its allelomorph. The results obtained thus far are shown in Table 1.

It is evident that all the matings except mating 14 are in accordance with the theory of inheritance previously stated, and for this reason no further comment need be made on them. Mating 14 requires special mention because of the excess of dark-eyed and the deficiency of pink-eyed, and to some extent of salmon-eyed, offspring produced. Up to the present no explanation that has occurred to us has been found satisfactory. Instead of making further attempts to find satisfactory explanations, we are planning to repeat the cross, using parents that are homozygous for black or chocolate, thereby making more certain of the exact genetic composition of the offspring produced and this in turn checking up on the genetic composition of the parents.

When Table 1 is considered merely from the standpoint of the monohybrid crosses involved, a number of unusual results are obtained. For instance, if matings 3, 4, 6, 7, 10 and 14 are combined to show the *Sm sm* × *sm sm* cross, it is found that there are 87 dark-eyed and only 42 salmon-eyed offspring produced. When this is ana-

A Tabulation of the Various Matings involving Salmon-eye (*sm*) and its Allelomorph and Pink-eye (*p*) and its Allelomorph. The Numbers in Parentheses indicate the Expected Results

Matings	Dark-eyed	Offspring Salmon-eyed	Pink-eyed	P
(1) PP Sm Sm × Pp sm sm	16(16)			
(2) PP Sm sm × PP Sm sm	19(15.75)	2(5.25)		
(3) PP Sm sm × PP sm sm	10(7.5)	5(7.5)		
(4) PP Sm sm × Pp sm sm	5(5.5)	6(5.5)		
(5) Pp Sm sm × Pp Sm sm	9(10.7)	1(3.6)	9(4.7)	.048181
(6) Pp Sm sm × PP sm sm	9(6.5)	4(6.5)		
(7) Pp Sm sm × Pp sm sm	20(11.6)	6(11.6)	5(7.8)	.010764
(8) PP sm sm × PP sm sm		20(20)		
(9) PP sm sm × pp Sm Sm	28(28)			
(10) PP sm sm × pp Sm sm	2(2)	2(2)		
(11) PP sm sm × pp sm sm		4(4)		
(12) pp Sm Sm × Pp sm sm	19(17)		15(17)	
(13) Pp sm sm × Pp sm sm		43(42.75)	14(14.25)	
(14) Pp sm sm × pp Sm sm	41(19)	13(19)	22(38)	.000000
(15) Pp sm sm × pp sm sm		36(31.5)	27(31.5)	
(16) pp sm sm × pp sm sm			2(2)	

lyzed biometrically it is found that the $\frac{\text{Deviation}}{\text{P. E. difference}}$ is equal to 5.875, thus proving that a deviation from expectation as great as the one occurring could not be expected according to chance. In a similar manner, however, when matings 12, 14 and 15 are combined to show the *Pp* × *pp* cross, it is found that there are 109 offspring produced carrying *P* and only 64 pink-eyed offspring. In this case the $\frac{\text{Deviation}}{\text{P. E. difference}}$ is equal to 5.066, which again indicates that the deviation is too great to be expected according to chance. One is forced to conclude that if the above results tended to disprove the single unit explanation for the inheritance of salmon-eye it would have a similar effect with regard to pink-eye.

In the heterozygous by heterozygous matings on the other hand the results obtained are nearer to expectation. For example, when matings 2 and 5 are combined to make up the *Sm sm* × *Sm sm* cross, the dark-eyed offspring number 28 and the salmon-eyed only 3. Here

the $\frac{\text{Deviation}}{\text{P. E. difference}}$ is equal to 2.914, which is so low that it does not preclude the possibility of the deviation being due to chance. By combining matings 5, 7 and 13 it is possible to give the complete data on the $Pp \times Pp$ cross. Here the results are very close to expectation, there being 79 offspring carrying *P* and 28 that are pink-eyed.

Because of the aberrant ratios obtained in some matings, there seems to be evidence of some disturbing elements being present. We already know something concerning one of these, and it may be well at this time to give more of the details. The matter referred to is the entire absence of pigment in the eyes of certain salmon-eyed animals. This has been mentioned previously and the statement was made that these animals could be detected, if they were black or chocolate, because the black or chocolate hairs were not diluted. Two females of the above type, one pink-eyed with intense black hair and the other pink-eyed with intense chocolate hair, were mated to true pink-eyed black males, which consequently had dilute black hairs. Fifteen offspring were produced. Of these, six were dark-eyed and nine were true pink-eyed. The mating may be represented as follows:

Pink-eyed black (*pp Sm Sm*) × "pink-eyed" black (or chocolate) (*Pp sm sm*) = dark-eyed (*Pp Sm sm*) and pink-eyed (*pp Sm sm*).

It must be remembered that if the "pink-eyed" black (or chocolate) females mentioned above had been of the composition *pp sm sm* instead of *Pp sm sm* they would have been true pink-eyed because of the epistatic relation of the pink-eye factor to the salmon-eye, and their black (or chocolate) hairs would have been dilute instead of intense. In addition, they would not have had any dark-eyed offspring.

Some crosses have been made with the purpose in view of testing the linkage relations of salmon-eye and pink-eye. The double heterozygotes were produced by mating pink-eyed reds to salmon-eyed reds, thus obtaining dark-eyed reds. The latter were then mated to the double recessives. The mating may be represented in the following manner:

$$P\ sm\ p\ Sm \times pp\ sm\ sm = \begin{cases} \text{"non-cross overs"} \begin{cases} P\ sm\ p\ sm - \text{salmon-eyed} \\ p\ Sm\ p\ sm - \text{pink-eyed} \end{cases} \\ \text{"cross overs"} \begin{cases} P\ Sm\ p\ sm - \text{dark-eyed} \\ p\ sm\ p\ sm - \text{pink-eyed} \end{cases} \end{cases}$$

It will be noted that the dark-eyed offspring represent a "crossover" class and the salmon-eyed a "non-crossover." Pink-eyed animals should be found in both classes. If there were independent assortment there should be one dark-eyed: one salmon-eyed: two pink-eyed in the offspring. The actual results obtained thus far are meager, but they seem to indicate independent assortment. To date there have been four dark-eyed, five salmon-eyed and fifteen pink-eyed offspring produced when double heterozygotes (*P sm, p Sm*) were mated to double recessives. Further tests will be made later when homozygous black (or chocolate) double heterozygotes and double recessives have been produced. As stated in a previous paragraph the use of animals of this composition will make one more certain of the genotypic composition of the offspring.

Summary

Salmon-eye is due to a single gene (*sm*), which causes the eye to have a pinkish appearance due to the absence of all pigment from the iris with the exception of a small ring, varying in width and intensity, surrounding the pupil. Unlike the pink-eye gene (*p*) it has no diluting effect on either black or chocolate pigment in the hair, but like the pink-eye gene it has no effect on red hair. Occasionally salmon-eyed animals are produced which do not have any pigment in the eyes, but the intensity of the black or chocolate hairs of these animals remains unaffected. So far as can be determined the salmon-eyed gene has no counterpart in any other species of animals. The pink-eye gene is epistatic to salmon-eye. The evidence obtained thus far indicates that these two factors are not linked.

Literature Cited

Castle, W. C.

1914. "Some new varieties of rats and guinea-pigs and their relation to problems of color inheritance," Am. Nat., Vol. 48, pp. 25–73.

NOTES ON LINKAGES IN MAIZE

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DURING the past several years many intercrosses were made between different varieties of maize in connection with the studies on the inheritance of white seedlings. Most of these varieties differed in genetic constitution in regard to several characters; this made it possible to use the same material for the study of linkage relation between some of them. As a result of that study the existence of linkages between several factors has been found.

Factors mentioned in this paper and their symbols are shown below.

C c	—a factor pair for aleurone color.	Complementary to *R r*
D_3 d_3	—dwarf 3	
Gm_2 gm_2	—germless seeds	
R r	—a factor pair for aleurone color.	Complementary to *C c*
Sh sh	—shrunken endosperm	
Su su	—sugary endosperm	
V_1 v_1	—virescent seedlings 1	
V_8 v_8	—virescent seedlings 8	
W_{11} w_{11}	—white seedlings 11	
Wx wx	—waxy endosperm	

Linkage between Sh sh and W_{11} w_{11}: A plant heterozygous for white seedling 11 having the genetic constitution W_{11} w_{11} Sh sh C c rr was crossed with another plant of the following constitution: W_{11} w_{11} sh sh C C RR. In the F_2 generation an indication of linkage between W_{11} w_{11} and Sh sh was observed, but since the F_2 data were not large enough for a definite conclusion the F_3 generation was grown in which both repulsion and coupling series were obtained, showing conclusively the existence of linkage between the C–c, Sh–sh and W_{11} w_{11} factors. The repulsion data for the linkage between Sh–sh and W_{11} w_{11} are given in Table I and the coupling data in Table II.

TABLE I

REPULSION DATA SHOWING THE LINKAGE BETWEEN *Sh–sh* AND W_{11}–w_{11}

Pedigree number	Sh W	Sh w	sh W	sh W
13,412–19	60	41	35	2
1,505– 3	116	40	45	3
– 4	167	65	41	7
– 5	84	32	24	3
– 8	60	15	16	1
Total	487	193	161	16

TABLE II

COUPLING DATA SHOWING THE LINKAGE BETWEEN *Sh–sh* AND W_{11}–w_{11}

Pedigree number	Sh W	Sh w	sh W	sh W
1,505–6	148	11	8	27
–7	172	15	17	40
Total	320	26	25	67

The percentage of crossing-over calculated by Yule's coefficient of association method (Collins, 1924) was found to be 31.25 for the repulsion and 13.40 for the coupling data. The difference between those two percentages is very large. It is known, however, that the percentage of crossing-over is not a fixed value, but is exposed to variations due to experimental errors, environmental and different genetic causes. As investigated by Collins (1924b) crossing-over for *C c* and *Wx wx* ranges from 10 per cent. to 45 per cent. In view of that, the difference found in the present case can not be considered extraordinary. Until the results of more extensive tests which are now in progress are available, the average of the two values obtained will be considered as the crossing-over value between Sh–sh and W_{11}–w_{11}. That average is 22.3 per cent.

Linkage between Sh–sh and D_3–d_3: In the F_2 generation grown from a cross between two plants having the genetic

constitutions D_3 D_3 sh sh C C R R and D_3 d_3 Sh Sh C c r r, respectively, a significant correlation between dwarf-3 and Sh–sh was observed. The data from the families which segregated dwarf-3 are given in Table III, and they show conclusively the existence of linkage between Sh–sh and D_3–d_3, the percentage of crossing-over being 22.75.[1]

TABLE III

F_2 DATA FROM A CROSS *Sh Sh*, d_3 d_3 × *sh sh*, D_3 D_3 SHOWING THE LINKAGE BETWEEN *Sh–sh* AND D_3–d_3

Pedigree number	Sh D	Sh d	sh D	sh d
1,457– 3	62	25	14	1
– 5	42	20	19	2
– 9	52	30	25	1
–12	53	23	26	1
–13	49	20	15	0
–15	30	22	21	2
–16	41	22	18	1
Total	329	162	138	8

Probable position of W_{11}–w_{11} *and* D_3 d_3 *in the first linkage group.* According to the last published map of the first linkage group (Demerec, 1924) four genes were located in it with the probable order C–sh–wx–v_1, crossing-over between C–sh being 4.3 per cent., between sh–wx, 21.8 and between wx–v_1, 7 per cent. Since it was found that crossing over between sh–w_{11} is about 22 per cent. and between sh–d_3, 23 per cent., it is to be expected that either or both of these factors will be located very close to Wx–wx or very far from it if their location is on the left side of C–c. As already stated above, the factor C–c in addition to Sh–sh was present in the crosses from which the data for linkage relations between sh–w_1, and sh–d_3 were obtained. From the data involving three factors located in the same chromosome it should be possible to determine the order of these three factors. Unfortunately, in both of the crosses the R–r factor, which is in

[1] All calculations of percentages of crossing-over are made by Yule's coefficient of association method (Collins, 1924a).

its effects complementary to C–c, was present also and made it impossible to separate C and c classes. Since it is known, however, that C–c and R–r are inherited independently, a regular distribution of R–r throughout the different classes is to be expected and a correction for R–r can be made. That has been done and the data thus corrected indicate that both W_{11}–w_{11} and D_3–d_3 are located to the right of Sh–sh. It is, however, necessary to confirm this indication with better data before it can be accepted as final.

Linkage between R–r and Gm_2–gm_2: Table IV gives the F_2 data or the crosses R R Gm_2 gm_2 × r r Gm_2 Gm_2 which shows that R–r and Gm_2–gm_2 are linked, crossing-over being 31 per cent.

TABLE IV

F_2 GENERATION DATA SHOWING THE LINKAGE BETWEEN *R–r* AND *Gm_2–gm_2* OBTAINED BY SELFING $\frac{R\ Gm_2}{r\ gm_2}$ PLANTS

Pedigree number	R Gm	R gm	r Gm	r gm
1,506– 1	204	68	94	5
– 2	111	35	35	6
– 3	180	71	69	11
– 4	236	84	124	2
– 5	242	93	103	15
– 6	139	51	62	2
– 7	165	53	66	4
– 8	67	26	22	2
– 9	136	72	50	12
–10	92	38	34	8
–12	151	61	81	5
–13	177	64	81	10
1,454– 8	92	23	32	1
–12	79	25	34	0
–13	78	21	51	0
–18	90	29	38	1
Total	2,239	784	976	84

As can be noted from Table IV the *gm* classes are significantly deficient, the difference from expectancy being 153 ± 18.7. The probable cause for that deficiency might be the fact that germless seeds sometimes develop

the germ and all which do that are classified in the Gm classes.

Linkage between Su–su and V_8–v_8: A family of sweet-corn which was heterozygous for virescent 8 has been extensively used in crosses with the white seedling material, and all the F_2 generation data resulting from those crosses show the linkage between Su–su and V_8–v_8. The data are given in Table V and the calculations indicate that crossing-over is 32.36 per cent.

TABLE V

F_2 DATA OBTAINED BY SELFING $\frac{su\ V_8}{Su\ V_8}$ PLANTS, SHOWING THE LINKAGE BETWEEN *Su–su* AND V_8–v_8

Pedigree number	Su V	Su v	su V	su v
1,457–5	74	9	8	12
1,469–6	110	26	25	18
1,476–5	127	23	11	16
1,490–2	106	29	24	15
–4	94	23	26	13
–5	67	21	20	11
–6	96	27	16	15
–7	83	22	16	13
1,491–3	183	34	33	35
Total	940	214	179	148

SUMMARY

The data are presented which show the linkage relations between the following factors:

W_{11}–w_{11} and Sh–sh with 22 per cent. crossing over,
D_3–d_3 and Sh–sh with 23 per cent. crossing over,
Gm_2–gm_2 and R–r with 31 per cent. crossing over, and
V_8–v_8 and Su–su with 33 per cent. of crossing over.

LITERATURE CITED

Collins, G. N.
1924a. Measurement of linkage values. *Jour. Agr. Res.*, 27: 881–891.
1924b. Factors influencing the rate of crossing-over in maize. (Abstract) *Anat. Rec.*, 29: 136.

Demerec, M.
1924. Genetic relations of five factor pairs for virescent seedlings in maize. *Cornell Agric. Exp. Sta. Mem.*, 84, p. 38.

THYSANOPTERA AND THE POLLINATION OF FLOWERS

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INTRODUCTION

THE problem of insect transmission of pollen is one which frequently arises in carrying on breeding tests and experimental work involving control of cross-pollination of flowers. During experimental breeding of sugar beets in Colorado during the years of 1920 and 1921, Thysanoptera of various species were noticed frequenting sugar beet flowers in numbers and moving freely from flower to flower. The possibility of pollen being carried and cross-pollination being secured in this way led to the experimental work which is reported in this paper. The larger part of the work was carried on at Stanford University but was completed at Fort Collins, Colorado, at the Colorado Agricultural College.

Thysanoptera or thrips are minute insects, for the most part winged, many species of which spend almost their entire adult and larval life in flowers of various species. These species of thrips feed on nectar and pollen and may be found crawling actively about in the corolla of almost any flower. They are extremely small, the species varying from .75 mm to 1.50 mm in length, and are well able to penetrate almost any covering used in breeding work. Unless paper bags, when they are used, are tied tightly around the stem, thrips can easily get in where it would be impossible for other insects to enter. They are very active and frequently fly from flower to flower of their own accord. The adults are fairly good fliers in still weather and may be carried some distance in a light wind. Although small in size, they often occur in large numbers and would be capable of carrying considerable pollen if each insect carried only a single grain.

IMPORTANCE AS POLLEN CARRIERS

Almost any flower picked during the summer will have at least one species of thrips in its corolla. By far the most numerous species in California during most of the year are, *Frankliniella tritici* (Fitch) and *Frankliniella occidentalis* (Pergande). These closely allied species may be found in practically any species of flowers from the first of May until hibernation in the fall. Their distribution is so general and they occur in such numbers that they, particularly the former, were used almost exclusively in the experiments reported in this paper. Other species also occur commonly but are not numerous enough to be of primary importance in bringing about pollination. Among the more common may be mentioned the following: *Orothrips kelloggii* Moulton, *Ankothrips robustus* Crawford, *Thrips madronii* Moulton, *Thrips magnus* Moulton, *Aeolothrips kuwanii* Crawford, *Thrips tabaci* Lindeman, *Sericothrips apteris* Daniel, *Sericothrips standfordii* Moulton, *Limothrips angulicornis* Bagnall, *Chirothrips insolitus* Hood, *Frankliniella minuta* (Moulton), *Taeniothrips inconsequens* Uzel.

The number of individuals present in the flowers as well as the amount of pollen per insect would determine, of course, the total amount of pollen that could be carried. In October, alfalfa flowers brought into the laboratory at Stanford yielded an average of forty-one thrips to the spike, the average per flower being about two. Some flowers had as many as fifteen thrips and some thrips carried as high as sixteen pollen grains. In March, one hundred plum flowers averaged three thrips to the flower, and California poppies (*Eschscholtzia californica*) averaged seven to the flower. *Clarkia elegans* averaged 5.5 thrips to the flower. In July, columbine flowers near Fort Collins, Colorado, had as high as fifty-five per flower.

As probably the greatest movement from flower to flower takes place on the wing, the question arose as to whether the pollen is shaken off in flying and as to how much pollen is actually carried from one flower to another. To obtain data on this point, thrips were caught

on the wing in an alfalfa field and the individuals were examined under the microscope for pollen grains. The average number of pollen grains per thrips for one hundred examined in this way was practically the same as for those taken directly from the alfalfa flowers (1.99 per thrips).

In an effort to determine to what extent Thysanoptera are capable of carrying pollen about with them from flower to flower, flowers containing thrips were brought into the laboratory where the insects were allowed to crawl out of them voluntarily. The thrips were then placed directly into alcohol and counts were made under the microscope of the pollen grains clinging to their bodies. The table given below summarizes the results obtained.

ABILITY OF THYSANOPTERA TO CARRY POLLEN

Flower	Species of Thrips	Number Examined	Largest Number Grains Per Thrips	Average Number Grains Per Thrips
Alfalfa	Frankliniella tritici	130	16	2.3 +
Acacia	Frankliniella tritici	8	1	1
Plum	Frankliniella tritici	16	3	.25
"	Taeniothrips inconsequens	16	4	1.25
Daisy	Frankliniella minuta	20	4	2
California poppy	Sericothrips apterus	10	3	3
" "	Frankliniella tritici	96	20	3.3
" "	Frankliniella minuta	3	3	2
Filaree	Frankliniella tritici	6	0	0
English laurel	Taeniothrips inconsequens	1	19	19
" "	Frankliniella tritici	12	6	4
Spirea	Frankliniella tritici	18	0	0
Lupine	Frankliniella tritici	19	76	26.5
Lilac	Frankliniella tritici	3	21	14
"	Thrips madronii	2	7	6
"	Aeolothrips kuwanii	1	13	13
Clarkia elegans	Frankliniella tritici	67	11	1.5
	Total number examined.....	428		

It appears that some kinds of pollen are much more readily carried than are other kinds. Thrips taken from lupines, for example, averaged 26.5 pollen grains, while thrips of the same species averaged only 3.3 pollen grains when taken from California poppies. This appears to be

due to the character of the pollen. The lupine pollen is very viscous, adhering to any part of the body in masses. The poppy pollen adheres only when held by bristles on the body or in the fringes of the wings and is never in masses. The Clarkia pollen is also viscous, but it is of large size and thus is not so readily carried. The grains are nearly as large as the heads of the insects. In spite of this, thrips may be seen dragging Clarkia pollen grains about with them attached to the feet and legs. In all likelihood, plants with this type of pollen, other factors being equal, stand a greater chance of being pollinated by thrips than do others, as pollen adheres where it will be most liable to come in contact with the stigmas of other flowers. Unless the pollen is viscous, it is rarely found on the ventral side of the insects. It is more abundant on the bristles of the thorax, the tip of the abdomen and in the fringes of the wings. Sometimes, it may be found clinging about the mouth parts. Those species of Thysanoptera having heavy well-developed bristles are better able to carry pollen grains on the body. It is not at all uncommon to find a thrips from one kind of flower carrying pollen of another kind of plant on its body.

That Thysanoptera do move from flower to flower is attested to by the fact that they have a very distinct seasonal movement. When plums were in bloom, the flowers were infested with at least seven species, while neighboring poppies were almost devoid of thrips, whereas they had previously been fairly well populated. The same movement took place when the lilacs came into bloom and again when the alfalfa bloomed. Apparently the thrips for a considerable area around congregated on these plants, causing a fairly heavy infestation. Certain plants, particularly those with a perfume, seem to be more attractive and movement takes place from one plant to another in numbers. This habit is of considerable economic importance, in addition to offering evidence of their ability to change their location with ease.

In order to be certain that pollen grains actually are shaken from the body and deposited on the stigmatic surface of flower pistils, California poppies (*Eschscholt-*

zia californica) were brought into the laboratory before they were pollinated but after the stigmas were receptive. Thrips carrying pollen were then taken from poppies whose anthers had dehisced, and were placed by means of a moistened needle on the stigmatic surfaces of the unpollinated poppies. While observing the insects through the binocular microscope the pollen grains could be readily seen clinging to their bodies. The thrips frequently stopped while wandering about and combed the wing fringes. During this process pollen was shaken free and was left adhering to the stigmas. Pollen grains were also dropped while the thrips were merely wandering about. Eight thrips were observed to leave the following number of pollen grains: No. 1 left 3; No. 2, 4; No. 3, 2; No. 4, 8; No. 5, 1; No. 6, 1; No. 7, 3; and No. 8, none. In no case did the thrips remain longer than four minutes on the stigmas.

A number of field experiments with lupines, alfalfa and poppies were carried out to determine whether thrips were actually able to bring about cross-pollination in the field. Flowers were covered with small paper tubes about four inches in length and one and a quarter inches in diameter. The tubes were closed around the stem below the flowers by tying with a thread, after first wrapping some cotton around the stem to make closure more complete and to prevent cutting the stem with the thread. The top of the tube was then folded over twice and secured with a small paper clip. In tests this type of covering was found to exclude all thrips, except in a few cases when the thread was tied too loosely or the cotton was not wrapped completely around the stem. Thrips, carrying pollen, were introduced on the point of a moistened needle and allowed to crawl off inside the tubes.

In the case of alfalfa and lupines very few flowers were pollinated by the pollen-carrying thrips introduced into the tube. In the case of both of these flowers the stigma had to be disturbed from its normal position in order to remove the anthers to prevent self-pollination, which occurs in both cases otherwise. The stigmas, which pre-

sent only a very small area for the reception of the pollen, were left without a surrounding envelope of petals, and the chance of thrips coming in contact with them was thus greatly reduced. All the thrips introduced carried quantities of pollen on their bodies.

The poppy has a large stigmatic surface and pollination took place in a large percentage of the cases. The stigmas are filiform, have the delicate papillae extending the whole length and are large enough to afford a ready foothold for the tiny thrips. The seeds are numerous and are borne in a one-celled capsule which was examined in each case to determine the number of seeds which set. As each required one pollen grain, this gave the minimum number left on the stigmas by the thrips. Out of 704 castrated poppies which had thrips from other poppies placed on them, 400 or 56.8 per cent. produced seed. The number of seeds per capsule varied from one to forty-eight. They commonly ranged from one seed to eight, anything above ten being the exception. A large series of checks was run to guard against faulty castration or exclusion of pollen as largely as possible.

SUMMARY OF RESULTS

The results of the work done show that thrips with habits conducive to cross-pollination are able to carry pollen grains of a variety of flowers by flight in sufficient quantities to be of considerable importance in pollination. Their ability to carry pollen from anthers to stigma depends on: (a) the size of the pollen grains; (b) the viscosity of the grains; (c) the attractiveness and (d) the number and species of thrips present. They are capable of bringing about cross-pollination of some species of flowers under field conditions in spite of their well-known tendency to "blight" flowers by sucking juices from the ovary and by the destruction of other tender parts of the flower, including the destruction of pollen grains.

The results obtained emphasize the importance of excluding thrips in breeding experiments where it is desired to prevent cross-pollination.

MENDELIAN FACTORS PRODUCING SELECTIVE FERTILIZATION

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ONE of the fundamental assumptions of the laws of Mendelism is the theory of the free combination of all gametes of opposite sex. It forms the basis for the chance distribution of all the possible gene combinations. We have recently become acquainted with cases, however, where the attraction between two gametes of opposite sex does not depend upon this sex differentiation alone but is affected also by other Mendelian factors. Such cases are now found in very different groups throughout the plant kingdom, in Basidiomycetes, in Dicotyledons and in Monocotyledons. It can hardly be doubted that such a selection of viable gametes in some combinations is a rather general phenomenon causing deviations from expected ratios. As in cases of linkage and of elimination of gametes or zygotes having a certain constitution, it causes an excess of some combinations and a deficiency of others in the offspring. A peculiarity which it has in common with heterogamy is in causing a difference between reciprocal crosses in higher plants. There is a selection between the male gametes only.

The first carefully analyzed case was published by Correns (1917, 1918, 1921). He investigated the sex inheritance in the dioecious *Lychnis dioica* L. (*Melandrium rubrum* Garcke) and *L. alba* Mill. (*Melandrium album* Garcke). The male plants are interpreted as heterozygous (Ff) and produce two types of pollen grains; male determining (f) and female determining (F). The female plants are homozygous (FF) and form only one

[1] Contributions from the Laboratory of Genetics, Bussey Institution of Harvard University.

type of eggs (F). In the F_1 generation of a normal cross (FF × Ff) Correns does not obtain the expected sex ratio 1 FF : 1 Ff, but a deficiency of males (Ff). He was able to show that the shortage of males (Ff) was due to a slower rate of growth of the male determiners f as compared with the female determiners F.

More recently Correns (1922) has investigated the sex inheritance of the dioecious *Rumex acetosa* L., where again the male plants are the heterozygotes (Ff) and the female plants the homozygotes (FF). As before, there appeared in the offspring of a normal cross FF × Ff an excess of the females (FF) which was due to a favoring of the female determiners F.

In both of Correns's cases, therefore, it is the pollen with the constitution F which was better able to function in the tissue of the female plant (FF) than the pollen type f.

Jones (1924) found a similar case in a cross between pop corn (SuSu) and sweet corn (susu). In the offspring of certain F_1 combinations an excess of Su plants appeared. In other crosses the expected ratio was found. A significant deviation appeared only when the pollen of the heterozygous Susu plant was used, and probably was due to the fact that in this pollen mixture the Su pollen was better able to function than the su pollen. Further, the difference in the behavior of the two pollen types was found only when females were used which carried also the Su factor, either in the homozygous or the heterozygous state. Of the three possible crosses with Susu pollen, a significant deviation appeared only in the cross Susu × Susu and the cross SuSu × Susu, but not in the cross susu × Susu, where the deviation was, according to Jones, only 1.88 times the probable error.

From these three reported cases one could draw the general conclusion that like tends to mate with like. In *Lychnis* and *Rumex* the F pollen is favored in a FF plant, in Zea the Su pollen is favored in plants of the constitution Susu and SuSu which carry also the Su factor.

The mutual attraction between like factors, however, is not a general rule. Heribert-Nilsson found an excess of red-nerved plants in the offspring of crosses made with the pollen of a heterozygous red-nerved *Oenothera Lamarckiana* of the constitution Rr on either heterozygous red-nerved plants (Rr) or homozygous white-nerved plants (rr); the homozygous red-nerved plants (RR) could not be investigated because the combination is lethal. Heribert-Nilsson was able, through a very simple method, to show (1923) that this excess was due to a slower rate of growth of the red-nerve determining pollen-tube r in the tissue of a Rr plant or a rr plant. The r tube is less likely to fertilize eggs than the R pollen-tube. In other words, unlike tends to mate with unlike.

In other Oenothera crosses, Renner (1919, 1921) explains deviations from the expected segregation also by the assumption of a selective pollen-tube growth.

Another case of a selection of gametes according to their constitution was recently published by East and Mangelsdorf (1925) as a result of the senior author's intensive studies on self-sterility in Nicotiana. They found special sterility factors acting in such a manner that pollen-tubes carrying a factor identical with a factor of the tissue in which they were growing failed normally to fertilize eggs. These sterility factors form a series of multiple allelomorphs of which three factors s_1, s_2 and s_3 have as yet been investigated in detail. For instance, normally in the offspring of a cross of a s_1s_2 plant with a s_1s_3 plant only the s_1s_3 and the s_2s_3 combinations appeared, s_1 pollen having failed to function on a s_1s_2 plant. Similarly, in the cross $s_1s_2 \times s_2s_3$ the combinations with s_2 pollen were lacking; while in the cross $s_2s_3 \times s_1s_3$ the s_3 pollen was deficient. The pollen of a homozygous plant functioned only on plants which did not contain the same factor. For instance, s_1s_1 pollen failed on s_1s_1, s_1s_2 and s_1s_3 plants, but not on s_2s_3 plants, and so forth.

These results are not always so clear as we have indicated. When bud pollinations are made, they are com-

plicated by what has been called pseudo-fertility, where some small portion of the inhibited pollen-tubes function and produce plants in the offspring. In such cases we have not a complete absence of expected segregates, but a deficiency only, as in *Lychnis, Rumex, Oenothera* and *Zea.*

It is very interesting that an identical situation has been found by Kniep and Brunswik in Basidiomycetes. The apparent differences are from the pure genetical viewpoint superficial. They are due to the diversity in the ontogenetical development of the fungi and the higher plants.

The diploid sporophore is homologous with the diploid sporophyte in the higher plants, and forms spores following a normal reduction division. After germination these spores form haplomycelia. Through some kind of fertilization the diploid state can be restored and the diplomycelium may form again a sporophore. The cycle is then closed.

In the Phycomycetes where the alternation of generations is similar, Blakeslee and his coworkers and also Burgeff have shown that in some species the haplomycelia are homothallic or monoecious, as one would call it in diploid plants; in other species they are heterothallic. One finds then plus mycelia and minus mycelia, both produced in the reduction division in equal numbers and therefore based on the segregation of a single pair of simple alleolomorphs. Every plus mycelium can mate with every minus mycelium, whatever the origin may be.

In the Basidiomycetes Brunswik found homothallic forms, while Kniep and Brunswik investigated other species which seemed to be heterothallic. But this heterothally is not, at least not in all species, the same as in the Phycomycetes. In some forms two groups of spores and therefore of haplomycelia are formed through the reduction division, only haplomycelia of different group mating with other. But in other species four groups appear showing rather complicated sex relations. The

situation is easily explained however with the assumption of two pairs of allelomorphs which are not linked and appear therefore after the reduction division in four haploid combinations in equal numbers. Only mycelia different in both loci can mate. For instance, a diplomycelium $a_1a_2b_1b_2$ forms the following four types of spores: a_1b_1, a_1b_2, a_2b_1 and a_2b_2. Of these four types only two pairs are absolutely different and can mate with each other: a_1b_1 with a_2b_2 and a_1b_2 with a_2b_1. If the original diplomycelium was obtained through the cross $a_1b_1 \times a_2b_2$, only the following two of the haplomycelia can mate with one of the parents: a_1b_1 with the parent a_2b_2 and a_2b_2 with the parent a_1b_1. All the other combinations are sterile because the haplomycelia have at least one factor in common.

In several of the species investigated the two or four groups formed by different diplomycelia are not identical as in the Phycomycetes. This difference is due, as first found by Kniep (1922), to the fact that we are dealing not with simple allelomorphs but with a series of allelomorphs. These series can be very large. Brunswik isolated 27 haplomycelia in one case (*Coprinus fimetarius* (L.) Fr.), all different from each other in two loci.

We see therefore in the *Basidiomycetes* as in *Nicotiana* multiple factors producing the effect that only gametes bearing different factors usually can mate. Again unlike tends to mate with unlike.

All the cases reported above seem to justify the following conclusion: *Mendelian factors exist which produce a selection among the gametes of a plant heterozygous for these factors; and both the mating of like with like or of unlike with unlike may be favored.*

Besides the cases reported above other data have been published which attempt to show a selective fertilization. These cases are doubtful, however, either because the number of plants investigated is too small or for other reasons. Selective fertilization should not merely be a method to explain unexpected segregations. It is a concrete theory susceptible of test in nearly every case.

While in a case of linkage between genes the deviation from the expected segregation is the same in reciprocal crosses, a selection takes place only between the pollen types. The eggs behave normally. The difference between the elimination of gametes and selective fertilization lies in the fact that the first is fixed for a given plant, the latter appears only in particular combinations. The gametes here are always potentially functional and fail in certain combinations only. *The selection is due to an interaction between the genotype of the female diploid plant and the male gametophyte in higher plants or between the two gametes in the Basidiomycetes.*

This interaction has been proved in the *Basidiomycetes* and in *Nicotiana,* where gametes of the same constitution do not mate; and in the cross between pop corn and sweet corn, where the stimulation of the Su pollen is found only when the Su factor is also present in the female plant. It was not susceptible of proof in *Lychnis* and *Rumex* because the female plants have always the same constitution, and in the red-nerved *Oenothera* in which the homozygous red-nerved plants are lethal and the heterozygous red-nerved and the white-nerved plants alone can be investigated. In the latter case we can assume by analogy with the experiments on other plants that it is the selective action of the r factor present in the male and the female plants of all crosses that produce the change in the ratio.

Further, in higher plants one may assume that *it makes a difference whether the factor for selective fertilization is present in the female in the homozygous or the heterozygous condition.* In the first case one would expect a more stringent selection. In species where a deficiency of some segregates is the result of the selection of gametes, in the plant homozygous for the selective factor, the deviation from the expected segregation should be higher than in a heterozygous plant.

In maize, using the data of Jones, the deficiency of the homozygous recessives in the cross Susu $\times$ Susu is 8.8 per cent. (probable error $\pm$ 0.45 per cent.), and in the

cross SuSu × Susu the deficiency is 21.6 per cent. (probable error ± 0.95 per cent.). The difference between the two deviations is 12.8 per cent. ± 1.02 per cent.

In the red-nerved Oenothera we find in the data of Heribert-Nilsson a deficiency of the homozygous recessive white-nerved plants of 12.7 per cent. (probable error ± 1.69 per cent.) in the cross Rr × Rr and a deficiency of 23.2 per cent. (probable error ± 1.81 per cent.) in the cross rr × Rr. The difference is 10.5 per cent. ± 2.88 per cent.

In both cases the deviation from the expectation is higher in the homozygous female than in the heterozygous. The difference between the two deviations is more than three times the probable error and therefore significant.

Finally, two general features of the behavior of the factors for selective fertilization should be mentioned because they are important from the viewpoint of genetical theory.

The degree of the selection varies greatly phenotypically as well as genotypically. The individual sets of observations of Correns, Jones and of Heribert-Nilsson show a variability which is higher than if due to chance alone and may in part be phenotypical, in part be caused by modifying genes. In self-sterile plants pseudo-fertility is known as a general phenomenon (Stout). East and Park (1917) found in Nicotiana an end-season-pseudo-fertility. In some species of Coprinus Brunswik found cases of a very clear pseudo-fertility where haplomycelia which should not mate fertilize each other to some extent.

The factors for selective fertilization themselves show in some cases a relatively high mutability. Such a mutability seems to be an explanation for the appearance of a large series of multiple allelomorphs, as found in the Basidiomycetes in several species. Kniep (1923) was able to detect in the Basidiomycete *Aleurodiscus polygonius,* which has two series of allelomorphs, in relatively small numbers of plants mutations in all four

allelomorphs of an original diplomycelium. The figures are however too small to calculate the mutation percentage.

BIBLIOGRAPHY

Brink, R. A. and J. H. Gillivard.
1924. Segregation for the waxy character in maize pollen and differential development of the male gametophyte. *Am. Journal of Bot.*, XI, p. 465–470.

Brunswik, H.
1924. Untersuchungen über die Geschlechts- und Kernverhältnisse der Hymenomycetengattung Coprinus. *Botan. Abhandl.*, V, 152 pp.

Buchholz, I. T., and A. F. Blakeslee.
1922. Studies of the pollen-tubes and abortive ovules of the globe mutant of Datura. *Science*, 55, pp. 597–599.

Burgeff, H.
1924. Untersuchungen über Sexualität und Parasitismus bei Mucorineen I. *Botan. Abhandl.*, IV, 135 pp.

Correns, C.
1917. Ein Fall experimenteller Verschiebung des Geschlechtsverhältnisses. Sitzungsber. d. preussischen Akad. d. Wiss., LI, p. 658–717.
1918. Fortsetzung der Versuche zur experimentellen Verschiebung des Geschlechtsverhältnisses. *Ibid.*, p. 1175–1200.
1921. Zweite Fortsetzung der Versuche zur experimentellen Verschiebung des Geschlechtsverhältnisses. *Ibid.*, XVIII, pp. 330–354.
1922. Geschlechtsbestimmung und Zahlenverhältnis der Geschlechter beim Sauerampfer (*Rumex acetosa*). *Biolog. Zentralbl.*, 42, pp. 465–480.

DeVries, H.
1924. Preferential Fertilization in *Oenothera Lamarckiana*. *Bot. Gaz.*, 77, pp. 73–80.

East, E. M., and J. B. Park.
1917. Studies on self-sterility I. The behavior of self-sterile plants. *Genetics*, II, pp. 509–609.

East, E. M., and A. J. Mangelsdorf.
1925. A new interpretation of the hereditary behavior of self-sterile plants. Proc. of the Nat. Acad. of Science, XI, pp. 166–171.

Heribert-Nilsson, H.
1920. Zuwachsgeschwindigkeit der Pollenschläuche und gestörte Mendelzaheln bei *Oenothera Lamarckiana*. *Hereditas*, I, pp. 40–67.
1923. Zertationsversuche mit Durchschneidung des Griffels bei *Oenothera Lamarckiana*.. *Ibid.*, IV, pp. 177–190.

Jones, D. F.
1924. Selective fertilization among the gametes from the same individuals. Proc. of the Nat. Acad. of Science, X, pp. 218–221.

Kniep, H.

1922. Über Geschlechtsbestimmung und Reduktionsteilung (Untersuchungen an Basidiomyceten). *Verhandl. d. Phys.-Med. Gesellschaft zu Würzburg*, N. F. 47, pp. 1–29.

1923. Über erbliche Änderungen von Geschlechtsfaktoren bei Pilzen. *Zeitschr. f. ind. Abstamm. u. Vererbungslehre*, XXXI, pp. 169–183.

Renner, O.

1919. Zur Biologie und Morphologie der männlichen Haplonten einiger Oenotheren. *Zeitschr. f. Botan.*, XI, pp. 306–380.

1921. Das Rotnervenmerkmal der Oenotheren. *Ber. d. deutsch. botan. Gesellsch.*, 39, pp. 264–270.

Stout, A. B.

1916. Self- and Cross-pollinations in *Cichorium intybus* with reference to sterility. Memoirs of the New York Botanical Garden, VI, pp. 333–454.

Ubisch, G. v.

1922. Abweichungen vom mechanischen Geschlechtsverhältnis. *Biolog. Zentralbl.*, 42, pp. 112–118.

SHORTER ARTICLES AND DISCUSSION

INDUCED CROSSING-OVER VARIATION IN THE X-CHROMOSOME OF DROSOPHILA

It has been shown by the author[1] that, although doses of X-rays up to 54 Holzknecht units cause an increase in the frequency of crossing over in the central regions of both of the long, centrally attached, V-shaped autosomes of Drosophila, they produce no or only a very slight effect (decrease?) in the distal regions. These results paralleled the already known effects of temperature found by Plough[2, 3] and of age found by Bridges[4] in one or more of the autosomes. In studying the cause of this peculiar regional differentiation, it would seem that results obtained with the X-chromosome might throw light on the problem, since the latter has the spindle fiber attached at the end (the "right hand" end, has now been proved by L. V. Morgan[5] and by Anderson[6]), but is not bent there. Thus it might be expected, if the point of attachment somehow determined the susceptibility to crossing-over variation, that most of the X-chromosome would respond to the above agents as do the distal regions of the long autosomes; the attached end of the X might or might not respond like their central regions, depending upon whether the bend in the autosomes, or other peculiarities due to a central attachment, are essential for the effect. It was indeed found by Plough and by Bridges that temperature and age fail to affect the greater part of the X, though they did not study the terminal regions. Mavor, however, found that X-rays did affect the X (again the terminal

[1] Muller, H. J., 1925, "The Regionally Differential Effect of X-Rays on Crossing over in Autosomes of Drosophila." *Genetics*, 10: 403–418.

[2] Plough, H. H., 1917, "The Effect of Temperature on Crossing over in Drosophila." *Jour. Exp. Zool.*, 24: 147–209.

[3] Plough, H. H., 1921, "Further Studies on the Effect of Temperature on Crossing over." *Jour. Exp. Zool.*, 32: 187–202.

[4] Bridges, C. B., 1915, "A Linkage Variation in Drosophila." *Jour. Exp. Zool.*, 19: 1–21.

[5] Morgan, L. V., 1925, "Polyploidy in *Drosophila melanogaster* with Two Attached X-chromosomes." *Genetics*, 10: 148–179.

[6] Anderson, E. G., 1925, "Crossing over in a Case of Attached X-chromosomes in *Drosophila melanogaster*." *Genetics*, 10: 403–418.

portions were not studied), causing a marked decrease in crossing-over frequency. This result, in comparison with the author's results with X-rays on the autosomes, seems to put the X in a class by itself, making it not comparable to the other chromosomes. If so, results from it would not be so pertinent to a solution of the problem concerning the autosomes.

In view of the above considerations, the author has attempted a partial repetition of the X-ray experiments on the X, and their extension to other regions of the X. The preliminary data are given here, as they tend to reconcile the results on the X with those on the autosomes, and it may be a considerable time before larger numbers can be obtained. The preliminary experiment involved the short extreme "left-hand" region, from scute to the apricot (white eye allelomorph) locus, the long section from apricot to bar, and a short "right-hand" section from bar to "beadex" (wing). Unfortunately the most extreme right region (beadex to bobbed) was not studied; this would have involved more complicated stock as bobbed males appear normal, and since at that time the left end was thought more probably to be the attached end, it was judged that bar-beadex might serve to represent the right region. The cross was as follows: females containing the mutant genes for scute and apricot in one X and those for white, bar and beadex in the other were back-crossed to scute apricot males. Only the sons could be classified with certainty for all four loci, but the females (not counted in all bottles) were reliable for scute and bar at least. Seven mothers served as controls, twelve mothers (sisters of these) were treated for fifteen minutes with X-rays from a current of fifty thousand volts and four milliamperes, at a distance of 16 cm from the target (54 H). The flies counted were derived from eggs laid from the fourth to the tenth day after raying. The results in brief were:

	Males + females			Males only						
	Scute-bar crossovers		Total counted	Scute apricot crossovers		Apricot-bar crossovers		Bar-beadex crossovers		Total counted
	Number	%		Number	%	Number	%	Number	%	
X-rayed	410	44.5	922	15	2.3	299	46.5	14	2.2	644
Controls	414	46.7	883	16	3.5	212	45.8	5	1.1	462

It will be seen that, although the numbers here are as yet comparatively small, the results differ significantly from Mavor's over a similar period and with a similar dose, for there could have been no such considerable decrease in crossing over in the central region as he found—unless, indeed, there was a decrease in the white-miniature region (studied by him) and a "compensatory" increase in the miniature-bar region. Although apricot-bar and scute-bar are long distances, so that the percentage of "cross-overs" (really, of recombinations) does not include all cases of crossing over in this region, nevertheless in previous work we have never found double crossing over in this region of the X frequent enough to obscure any considerable decrease of the total crossing over such as Mavor found. Very large counts are necessary to obtain reliable estimates of coincidence; the values for X-rayed and control lots obtained in the present experiments, so far as they go, show no significant differences from the values normally obtained or from each other.

It is not intended here to question the accuracy of Mavor's extensive results, but merely to show that under certain conditions, with given stocks and crosses, effects of X-rays may be obtained over the greater part of the X-chromosome which sensibly agree with those found by the present author in those parts of the autosomes not near the spindle fiber. Possibly too, then, further work with the autosomes might reveal in those regions under some circumstances a significant decrease in crossing over caused by X-rays, similar to what Mavor found in the X. This will require investigation, with the employment of larger doses of rays; there was, however, a suggestion of such effects in the results already reported for the autosomes.

As for the effects on other parts of the X than those previously investigated in this connection, it is interesting to note that the extreme left end—furthest from the attachment—did show an apparent decrease. On the other hand, the results, if taken at their face value, certainly suggest strongly an actual increase in crossing-over frequency in the right-hand region. This would be interesting, if borne out by larger numbers, in view of the location of the spindle fiber nearby, and the increases observed in the neighborhood of the spindle fiber in the long autosomes.

From the foregoing it is seen to be quite possible, or even likely, that the X-chromosome does not react to X-rays in a manner greatly different from the autosomes, *when regions are com-*

pared which correspond in their relation to the point of fiber attachment. If this is true, it would mean that the bend in the autosomes is not a causal agent in the effects, since the X is not thus bent. Beyond this, however, it would seem at the present time premature to speculate regarding the mechanism by which the spindle fiber might operate, in thus making the chromatin specially susceptible to variation in crossing over as a result of X-rays, and, presumably of heat, age and other non-genetic factors.

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ON THE INHERITANCE OF ALBINISM AND BROWN PIGMENTATION IN MICE

UP to the present time only one case of linkage has been reported in the house mouse. The color characters, pink-eye and albinism (with its allelomorphs), are involved. In the Norway rat two genes, apparently homologous with those of mice just mentioned, are linked with a third gene, red-eye (Castle and Wright, 1915; Castle, 1919; Dunn, 1920; Castle and Wachter, 1924; Detlefsen and Clemente, 1924, and Feldman, 1924). A linkage which produces about 13 per cent. crossing over exists in rabbits between a color pattern (English or Dutch) and Angora coat (Castle, 1924). Recently Castle has found in rabbits a rather loose linkage (41.1 per cent. crossing over) between brown pigmentation and albinism (or its allelomorphs) (Castle, 1924a).

This discovery raises an interesting question. Castle says:

> Analogous variations to those discussed in this paper, producing brown pigmentation and multiple albino allelomorphs, are known both in mice and in guinea-pigs, but in neither of these species has linkage been reported between brown and albinism. The matter is perhaps worthy of further investigation, although it is scarcely to be expected that completely homologous chromosomes exist in forms so widely separated systematically as rabbits, guinea-pigs and mice.

In an effort to obtain evidence upon this point, the present experiment was undertaken. A number of mice were produced which were homozygous for brown and for one of the albino allelomorphs, ruby-eyed dilution, described by the senior author

(Feldman, 1922). These double recessives were mated to animals possessing the dominant allelomorphs of both factors, namely, black and intense color. The resulting heterozygotes showed black, intense pigmentation and therefore resembled the dominant parent. Their genetic composition concerning these factors was *Cc*^{r} *Bb*. They were back-crossed to mice of the

TABLE 1

SUMMARY OF DATA CONCERNING THE GENETICS OF BROWN PIGMENTATION IN RELATION TO ALBINISM AND PINK-EYE IN MICE

Characters concerned		Observed class frequencies				Total	Source of data
Brown and Ruby-eye		CB	c^{r}B	Cb	c^{r}b		
	Numbers	129	140	134	118	521	New data
	Deviations from expected	− 1.25	+ 9.75	+ 3.75	− 12.25		
	Deviation / P. E.	.18	1.47	.57	1.83		
		BD	Bd	bD	bd		
Brown and Pink-eye	Numbers	942	900	956	936	3734	
	Deviations from expected	+ 8.5	− 33.5	+ 22.5	+ 2.5		Detlefsen and Roberts
	Deviation / P. E.	.48	1.89	1.26	.12		
		BD	Bd	bD	bd		
Brown and Pink-eye	Numbers	730	193	185	72	1180	
	Deviations from expected	+ 66.25	− 28.25	− 36.25	− 1.75		Little and Phillips
	Deviation / P. E.	5.82	3.15	4.05	.30		

NOTE: Since this paper went to press unpublished data secured by William H. Gates have been kindly loaned. In a back-cross involving brown- and pink-eye, 348 mice were recorded. Observed parental combinations were 160; observed recombinations were 188. The deviation was 2.22 times the probable error. These data agree fully with those of Table 1, and are further evidence that brown- and pink-eye are not linked in mice.

double recessive stock. Four classes of offspring resulted, which were easily classified when about ten days old.

We would expect equality of the four combinations if no linkage exists between the genes for brown and the albino allelomorphs. The heterozygous individuals would form gametes of the types *Cb, c'B, Cb, c'b* in approximately equal numbers. On the other hand, if the two genes in question are linked, the parental combinations, *CB* and *c'b*, would exceed the recombinations, *c'B* and *Cb*. The extent of the difference would depend on the strength of the coupling. Our results are presented in Table 1. No class of young showed a significant deviation from one quarter of the total. When the combined parental classes are compared with the combined non-parental or recombination classes, a difference of 27.0 ± 7.7 exists in favor of the non-parental group. The difference is contrary in sign to that which would be expected if linkage existed.

Albinism and pink-eye are known to be linked in mice, as already stated; therefore, data concerning a cross between brown and pink-eye should contribute to this question. Obviously, if albinism and brown are linked, the latter would also show linkage with pink-eye. Two sets of data concerning this point have been published. Those of Detlefsen and Roberts (1918) were secured from a back-cross of mice heterozygous for non-agouti, brown and pink-eye with the triple recessive, pink-eyed, non-agouti, brown animals. The data of Little and Phillips (1913) were obtained by mating *inter se* F_1 individuals obtained by a cross between a wild type mouse and dilute, pink-eyed, non-agouti browns.

Table 1 includes these data, rearranged for a consideration of the segregation of brown and pink-eye only. In the data of Detlefsen and Roberts the four groups, dark-eyed black, pink-eyed black, dark-eyed brown and pink-eyed brown, would be expected with approximately equal frequency if no linkage existed between brown and pink-eye. An examination of their data reveals no significant differences between the four classes. The combined parental classes exceed the non-parental by 22.0 ± 30.5, which can not be considered a significant difference. The data of Little and Phillips are not as satisfactory for our purpose. The gametic ratio of heterozygous individuals is not as clear when they are mated *inter se*, as when they are back-crossed to the ultimate recessive type. The segregation exhibited by this cross gives no indication of the linkage of brown with pink-eye. The excess of dominant classes is marked, however.

The black classes exceed the brown by 3.79 times the probable error, while the dark-eyed exceed the pink-eyed by 2.99 times the probable error. These deviations seem to be explained by a higher selective mortality of the recessive classes.

The original data of this paper concerning brown and albinism and data dealing with crosses involving brown and pink-eye reveal no evidence of the linkage of brown pigmentation with either albinism or pink-eye in mice. We must conclude, therefore, that one or both of the following situations exist: (1) The chromosome constitutions of rabbits and mice are sufficiently different to permit the linkage of brown with albinism in the one case and not in the other; (2) the brown variation in rabbits is not the homologue of the one which has been studied in mice.

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LITERATURE CITED

Castle, W. E.
1919. "Studies of heredity in rabbits, rats and mice," Carnegie Inst. Wash. Pub., 288.

Castle, W. E.
1924. "Linkage of Dutch, English and Angora in rabbits," Proc. Nat. Acad. Sci., 10, 107–108.

Castle, W. E.
1924a. "On the occurrence in rabbits of linkage in inheritance between albinism and brown pigmentation," Proc. Nat. Acad. Sci., 10, 486–488.

Castle, W. E., and Wachter, W. L.
1924. "Variations of linkage in rats and mice," *Genetics*, 9, 1–12.

Castle, W. E., and Wright, S.
1915. "Two color mutations of rats which show partial coupling," *Science*, N. S., 42, 193–195.

Detlefsen, J. A., and Clemente, L. S.
1924. "Linkage of a dilute color factor and dark-eye in mice," *Genetics*, 9, 247–260.

Detlefsen, J. A., and Roberts, E.
1918. "On a back cross in mice involving three allelomorphic pairs of characters," *Genetics*, 3, 573–598.

Dunn, L. C.
1920. "Linkage in mice and rats," *Genetics*, 5, 325–343.

Feldman, H. W.
1922. "A fourth allelomorph in the albino series in mice," AMER. NAT., 56, 573–574.

Feldman, H. W.
1924. "Linkage of albino allelomorphs in rats and mice," *Genetics*, 9, 487–492.

Little, C. C., and Phillips, J. C.
1913. "A cross involving four pairs of Mendelian characters in mice," AMER. NAT., 47, 760–762.

A GYNANDROMORPHIC BEE OF THE GENUS DIANTHIDIUM

On September 27, 1925, I took a number of specimens of *Dianthidium sayi* Ckll. on the flowers of *Sideranthus spinulosus* on the university campus. Among these was a very interesting gynandromorph. While a number of gynandromorphic bees have been found and recorded, as far as it can be ascertained, this is the first to be recorded from this genus.

The characters observed and noted were: Length, about ten mm; bilateral division shown in head and thorax, but no apparent division in the abdomen; male characters on the right side and female on the left; occiput on the right side black, with small yellow spot near cheeks; on left side black with broad, ferruginous band extending well into cheeks; right side of face yellow; left side yellow with reddish, coppery margins; supraclypeal area yellow on right side; black on the left; clypeus yellow on right; on left, mid-half black, rest yellow with tinge of red; right mandible yellow except distal end red; left mandible more massive; black, with distal portion red; right antenna thirteen-jointed; black; joints three and four with reddish spot above; distal joint slightly pointed; left antenna twelve-jointed; black, except joints three and four red; distal joint not pointed. Thorax: right side, anterior lateral region of mesonotum with yellow spot; left side with wide, reddish band extending to tubercles; scutellum black on right side, with small reddish spot; left half mostly red; axillae; black on right side; red on left. Right legs male; coxa of posterior leg with spine. Left legs female; no spine to posterior coxa. Abdomen, male; seven segments; markings and sculpturing similar on both sides; genital organs apparently male.

In going over a number of bees of this species, a considerable amount of variation was noted. The variation occurs in the extent of red and black in the markings on the head and abdomen of both males and females.

The extremes met with are given below. Many intermediate variations and combinations were found. As a rule, a greater amount of red or black found on a given structure was accompanied with a greater amount of red or black on many or all structures which had been found to show this variation. Characters and extent of variations on head of females; vertex, with

or without wide, continuous red band; front, with or without red spot; mandibles red or black; clypeus almost entirely black or red; scape red or black; joint two of antenna red or black (usually black); joints three and four red or black.

In the males the extremes noted were: antennae all black to scape and joints two, three and four all red; all segments of abdomen black, to first four segments red.

These differences do not appear to indicate geographical races, for groups were taken from a number of habitats and were found to vary, among themselves, in a similar manner. Another species of bees *Andrena prunorum* Ckll. has been found to vary in a comparable manner. The proportion of red and black on the head and thorax varies quite extensively within the species.

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GENES FOR THE EXTENSION OF BLACK PIGMENT IN THE CHICKEN

MR. D. G. STEELE, of the Genetics Department, University of Wisconsin, has very kindly called attention to a gross error in my note on "Genes for the extension of black pigment in the chicken," appearing in THE AMERICAN NATURALIST 57: 284–287. The sentence beginning at the bottom of page 284 reads: "In the F_2 the three expected classes appeared, though there was quite a deficiency in numbers in the Columbian class." And the next sentence: "The ratio secured was 25 white, 50 black and 13 Columbian, the theoretical expectation being 22–44–22."

These sentences should read: "In the F_2 the three expected classes appeared, the numbers being in fair agreement with theory. The numbers obtained were 50 blacks, 13 Columbians and 25 whites, the theoretical expectation being 49.5 : 15.2 : 22."

My interest at the moment was centered on Dunn's extension gene E^m and I inexcusably overlooked the fact that a dihybrid with an F_2 expectation of 9 : 3 : 4 was being dealt with. Since then my attention has been claimed by other matters and the blunder had not been noticed.

Attention may be called to a typographical error in the first line of the last paragraph on page 284. "The back crosses, F_1 ♀" should read: "The back crosses, F_1 ♂."

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